ROBUST SUMMARY
Algal growth inhibition assay

Test Substance	
Remarks	Isoprene (CAS No. 78-79-5) also known as 1,3-butadiene,2-methyl The test substance was stable for the duration of all the studies performed by the test house.
Method	
Method/guideline followed	OECD Guide-line 201and EC Directive 92/69 C3
GLP	Yes
Year	2002
Endpoint	Growth rate
Species	Pseudokirchneriella subcapitata (formerly Selenastrum capricornutum) (Algae)
Exposure period	96 hours
Limit test	No
Analytical monitoring	Yes
Study Design	Triplicate algal cultures, with an initial cell density of 1 x 104/ml, were exposed to Isoprene at nominal concentrations of 4.27, 9.39, 20.7, 45.5 and 100 mg/l. Because the test substance was known to be volatile, the test was conducted in completely filled and sealed vessels. The test media were prepared, either directly or by dilution, from an aqueous preparation which was made using techniques designed for generating a water accommodated fraction (WAF); the test substance was injected through a silicone septum into an amber glass vessel containing algal medium and was stirred for approximately 21 hours before aliquots were removed and used to provide the test media. The cultures were incubated in an orbital incubator under continuous illumination at temperatures ranging from 23.4 to 24.0°C for 96 hours. The exposure levels were monitored by measuring the concentrations of Isoprene in samples of the test media using a GC method of analysis. Cell numbers were measured daily to monitor growth, and the test results are expressed in terms of the area under the growth curve and growth rate.
Evaluation of data	The area under the growth curve is taken to be an index of growth and is calculated mathematically. The E_bC_{50} ("x"h) is the median effect concentration for inhibition of growth based on a comparison of areas under the growth curves after "x" hours. The E_bC_{50} was calculated by a computer program (Stephan:1977, 1982) using percentage effect and the nominal and measured test concentration in test samples. The "no observed effect concentrations" (NOEC) was determined using Dunnett's multicomparison test to compare the percentage inhibition in the test group with that for the control cultures

Results	(Dunnett:1955, 1964). The measured concentrations of Isoprene ranged between 14 and 39% of their nominal values at the start of the test and between 20 and 32% of nominal after 96 hours. Based on an arithmetric mean, the overall mean measured levels of Isoprene were 0.846, 1.68, 6.00, 10.3 and 35.2 mg/l Area under the growth curve (measured concentrations): EbC50 (72 h) : 15.3 mg/l (95% confidence limits, 12.9 & 18.6 mg/l) EbC50 (96 h) : 15.5 mg/l (95% confidence limits, 13.3 & 18.4 mg/l) No observed effect concentration (NOEC) : 1.68 mg/l Average specific growth rate: ErC50 (0 - 72h) : >35.2 mg/l (34% inhibition) ErC50 (0 - 96 h) : >35.2 mg/l (31% inhibition) No observed effect concentration (NOEC) : 6.00 mg/l
Observations	After 96 hours of exposure, approximately 25% of the algal cells at 10.3 mg/l and the majority of cells at 35.2 mg/l were swollen and/or mis-shapen.
Conclusions	After 72 and 96 hours of exposure to Isoprene, the E_bC_{50} values were 15.3 and 15.5 mg/l respectively; the E_rC_{50} values were >35.2 mg/l, with 34 and 31% inhibition respectively. The "no observed effect concentration" (NOEC) for area under the growth curve and growth rate respectively, were 1.68 and 6.00 mg/l.
Data Quality	Valid without restrictions
Reference:	Huntingdon Life Sciences Ltd. 2002. Algal growth inhibition assay. Project ID CSS 029 Huntingdon Life Sciences Ltd., Cambridgeshire, England
Other	
Last changed	15 May 2003 Robust summary prepared by contractor to Olefins Panel

ROBUST SUMMARY

Acute Toxicity to *Daphnia Magna*

Test Substance	
Remarks	Isoprene (CAS No. 78-79-5) also known as 1,3-butadiene,2-methyl The test substance was stable for the duration of all the studies performed by the test house.
Method	
Method/guideline followed	OECD Guide-line 202 and EC Directive 92/96 C2
GLP	Yes
Year	2002
Test type	Static
Species	*Daphnia magna* (Crustacea)
Exposure period	48 hours
Limit test	No
Analytical monitoring	Yes
Study Design	Groups of twenty Daphnia, less than 24 hours old, were exposed for 48 hours to Isoprene, prepared in Elendt M4 medium at nominal concentrations of 2.13, 4.70, 10.3, 22.7 and 50.0 mg/l. Because the test substance was known to be volatile, the test was conducted in completely filled and sealed vessels. The test media were prepared, either directly or by dilution, from an aqueous preparation which was made using techniques designed for generating a water accommodated fraction (WAF); the test substance was injected through a silicone septum into an amber glass vessel containing Elendt M4 medium and was stirred for approximately 22 hours before aliquots were removed and used to provide the test media. The exposure levels were monitored by measuring the concentrations of Isoprene in samples of the test media using a GC method of analysis. Observations of the Daphnia in each control and test vessel were made after 24 and 48 hours.
Evaluation of data	EC50 values were calculated using a computer program (Stephan's: 1977,1982) which uses the number of *Daphnia* exposed and the number immobile at each nominal and measured concentration. The "no observed effect concentration" (NOEC) was derived by direct inspection of the data on the immobility of the animals. An incidence of more than 10% is considered to be significant.
Results	Although lower than intended, the measured concentrations of Isoprene at the start (between 30 and 48% of their nominal values) were adequately maintained during the test, giving measured levels of between 30 and 51% of nominal after 48 hours. Based on an arithmetic mean, the overall mean measured levels of Isoprene were 0.648, 1.55, 3.52, 9.47 and 25.4 mg/l. After 48 hours, the lowest measured concentration resulting in 100%

Conclusions	immobility was 9.47 mg/l and the highest measured concentration at which no immobilisation occurred was 3.52 mg/l. 48-hour EC50 value = 5.77 mg/l (95% confidence limits of 3.52 and 9.47 mg/l)(measured concentration) NOEC = 3.52 mg/l (measured concentration)
Data Quality	Valid without restrictions
Reference:	Huntingdon Life Sciences Ltd. 2002. Acute Toxicity to *Daphnia Magna*. Project ID CSS 033 Huntingdon Life Sciences Ltd., Cambridgeshire, England
Other	
Last changed	15 May 2003 Robust summary prepared by contractor to Olefins Panel

ROBUST SUMMARY

Acute Toxicity to Rainbow Trout (Semistatic conditions)

Test Substance	
Remarks	Isoprene (CAS No. 78-79-5) also known as 1,3-butadiene,2-methyl The test substance was stable for the duration of all the studies performed by the test house.
Method	
Method/guideline followed	OECD Guide-line 203 and EC directive 92/96 C1
GLP	Yes
Year	2002
Test type	Semistatic
Species	*Oncorhynchus mykiss* (fresh water fish)
Exposure period	96 hours
Limit test	No
Analytical monitoring	Yes
Study Design	Groups of ten juvenile fish were exposed for 96 hours to Isoprene, prepared in diluent water (dechlorinated tap water, ca. 150-200 mg/l as CaCO3) at nominal concentrations of 2.13, 4.70, 10.3, 22.7 and 50.0 mg/l. Because the test substance was known to be volatile, the test was conducted in completely filled and sealed vessels. At each concentration, the test medium was prepared from an aqueous preparation which was made using techniques designed for generating a water accommodated fraction (WAF); the test substance was injected through a silicone bung into a glass vessel containing diluent water and was stirred for approximately 24 hours before aliquots were removed and used to fill duplicate test vessels at each concentration. The exposure levels were monitored by measuring the concentrations of Isoprene in samples of the test media using a GC method of analysis. The measured concentrations of Isoprene ranged between 42% and 105% of their nominal values in samples of freshly prepared media and between 44 and 84% % of their nominal values in samples of expired (24-hour-old) media (between 68 and 106% of their starting values). Based on a geometric mean, the overall mean measured levels of Isoprene were 1.68, 3.57, 6.71, 15.0 and 28.7 and 28.7 mg/l. Observations of the fish were made after 0.25, 2, 4, 24, 48, 72 and 96 hours of exposure.
Evaluation of data	LC50 values were estimated by a computer program (Stephan:1977,1982) using the number of fish exposed and the number dead at each measured concentration.
Results	After 96 hours, the highest measured concentration at which no mortality had occurred was 3.57 mg/l and the lowest at which there was 100% mortality was 15.0 mg/l. Treatment-related effects were exhibited at 6.71 mg/l and higher concentrations.

Conclusions	Based on these findings the following values have been estimated: 96-hour LC50 value = 7.43 mg/l (95% confidence limits of 6.71and 15.0 mg/l) NOEC = 3.57 mg/l (measured concentration) LC50 = 7.43 mg/l (measured concentration)
Data Quality	Valid without restrictions
Reference:	Huntingdon Life Sciences Ltd. 2002. Acute Toxicity to Rainbow Trout (Semi-static exposure conditions). Project ID CSS 032. Huntingdon Life Sciences Ltd., Cambridgeshire, England
Other	
Last changed	15 May 2003 Robust summary prepared by contractor to Olefins Panel

ROBUST SUMMARY

Assessment of Biodegradability using the closed bottle method

Test Substance	
Remarks	Isoprene (CAS No. 78-79-5) also known as 1,3-butadiene,2-methyl The test substance was stable for the duration of all studies performed bt the test house. The carbon content of the test substance was determined before the start of the Closed Bottle test using a CEC Model 440 Elemental Analyser. The measured carbon content (88%) was equivalent to 102% of the theoretical value (85.9%).
Method	
Method/guideline followed	OECD Guide-line 301D and EC Directive 92/69, C.4-E and EPA OPPTS 835.3110;
GLP	Yes
Year	2002
Type:	Aerobic
Inoculum:	Domestic sewage effluent
Concentration:	2mg/l related to test substance
Contact time:	28 days
Test inoculum preparation	Main test A sample of secondary effluent was collected on the day of the test from, a trickling-filter plant, which treats predominantly domestic waste. It was maintained under aerobic conditions in the laboratory, then, immediately before use, filtered through glass wool and the filtrate used as the inoculum for the test (1 ml filtrate/litre test medium).
Study design	Main test Eighteen bottles were filled with a mineral salts medium, inoculated with unacclimated sewage effluent at a loading of 1ml/l, and the test substane at a nominal loading of 2 mg/l. The dissolved oxygen (DO) concentration in replicate bottles was measured on Days 0, 5, 7, 11, 14, 18, 21, 25 and 28. Four bottles were established for a concurrent five-day microbial inhibition assay, in which the biodegradation of the readily biodegradable reference substance, sodium benzoate, was examined in the presence of the test substance. DO concentration was measured in replicate bottles on Days 0 and 5. A further eighteen bottles were filled with mineral salts, inoculated with unacclimatised sewage effluent at a loading of 1 ml/l, and the reference substance, sodium benzoate at a nominal loading of 5 mg/l. DO concentrations in replicate bottles was measured on Days 0, 5, 7, 11, 14, 18, 21, 25 and 28. All test systems were incubated at 22 +/- 2°C in darkness. Theoretical oxygen demands for the test and reference substances were based on their emperical formulae and molecular weights.

Results	The study was initiated on 13 March 2002. A maximum of 60% biodegradation was measured on Day 28 of the main Closed Bottle test. Biodegradation was not established in five BOD bottles on or after Day 18 of the test, but had occurred (54%) in the three remaining bottles measured on or after Day 18. The mean Total Viable Count of the sample of final sewage effluent in the main test was 7.1 x 105 Colony forming Units (CFU) per ml and the mean count in inoculated MSM on Day 0 was 3.3 x 104 cfu/ml. The presence of the test substance did not cause any inhibitory affect on the normal degradative activity of the microbial inoculum on the reference substance.
Study design	Supplementary investigation Eight bottles were established with mineral salts medium and an inoculum of medium (10 ml/l) from a BOD bottle incubated for 28 days as part of the main test (isoprene degradation in the BOD bottle used had achieved 60% on Day 28). Four bottles were of a modified design and were treated with isoprene (nominally 2 mg/l). Four bottles contained inoculum and mineral salts medium alone. The DO, pH and temperature of two bottles containing isoprene and two containing inoculum and mineral salts medium alone were analysed on Day 0 and following seven days of incubation in darkness at 22 ± 2°C. A maximum of 64% biodegradation was measured on Day 7 of the supplementary investigation. This confirmed that isoprene was biodegradable in the presence of a pre-exposed inoculum.
Degradation Kinetic of test substance	60 (±) % after 28 day(s) 5 day(s) 5 and 2 % 7 day(s) 4 and 4 % 11 day(s) 3 and 2 % 14 day(s) 5 and 5 % 18 day(s) 60 and 2 % 21 day(s) 7 and 54 % 25 day(s) 9 and 13% 28 day(s) 58 and 2 %
Control substance Kinetic	Benzoic acid, sodium salt 5 day(s) 79 and 78 % 28 day(s) 86 and 86 %
Conclusions	Biodegradable in the presence of pre-exposed inoculum
Data Quality	Valid without restriction
Reference:	Huntingdon Life Sciences Ltd.2003. Assessment of biodegradability using the closed bottle method Project ID CSS 036. Huntingdon Life Sciences Ltd.,

Other Last changed	Cambridgeshire, England. 15 May 2003 Robust summary prepared by contractor to Olefins Panel

Robust Summary

Biodegradation

Test Substance:	Isoprene, inhibited with 100 ppm p-tert-butylcatechol CAS No. 78-79-5: CAS Inventory Name: 1,3-Butadiene, 2-methyl-
Method/Guideline:	OECD Guideline 301F
Year (guideline):	1992
Type (test type):	Ready Biodegradability: Manometric Respirometry Test
GLP (Y/N):	Yes
Year (study performed):	2003
Inoculum:	Domestic activated sludge
Exposure Period:	28 Days
Test Conditions: • **Note: Concentration preparation, vessel type, replication, test conditions.**	Triplicate test systems were used to evaluate the biodegradability of the test and positive control substances at mean concentrations of 51mg/L and 46 mg/L, respectively. Blank test systems, which did not contain the test or positive control substance, were run concurrently in triplicate. The total suspended solids (TSS) of the activated sludge was determined to be 4.28 g/L. The inoculum was added at a 1% loading volume of sludge supernatant to test medium. The microbial count of the inoculum was 10^5 CFU/mL. One liter of test medium, which was aerated for 24 hours with carbon dioxide free air, was added to each one liter respirometer flask. The test substance was weighed in an air tight syringe and injected into the test medium. The test system was sealed immediately after addition of the test substance. An aliquot of the positive control stock solution was added to the appropriate test flasks. An unacclimated activated sludge inoculum was used in this study. The inoculum was obtained from the Clinton Sanitary Wastewater Treatment Plant, Annandale, NJ, USA. The treatment plant receives domestic sewage. All test systems were placed on a Coordinated Environmental Services (CES) automated respirometer which automatically recorded the oxygen uptake in general agreement with the OECD guideline. The 28-day study was conducted at a temperature range of 22 ± 1°C.

Results: **Units/Value:** **Note: Deviations from protocol or guideline analytical method.**	Biodegradation was based on oxygen consumption and the theoretical oxygen demand of the test substance as calculated using results of an elemental analysis of the test substance. By day 14, >60% biodegradation of positive control was observed, which meets the guideline requirement. No deviations from the protocol occurred that affected the integrity of the study data. The average percent biodegradation of the test substance was determined to be 61% on day 28. The test substance can be considered readily biodegradable. Sample: Test Substance; % Degradation* (day 28): 75, 55, 53; Mean % Degradation (day 28): 61 Sample: Na Benzoate; % Degradation* (day 28): 91, 100, 89; Mean % Degradation (day 28): 94 * replicate data
Conclusion:	Readily biodegradable
Reliability:	(1)-Reliable without restriction.
Reference:	ExxonMobil Biomedical Sciences, Inc. 2004. Ready Biodegradability: Manometric Respirometry test. Study # 177294A
Other (source):	Olefins Panel, American Chemistry Council

ROBUST SUMMARY

Algal growth inhibition assay

Test Substance	
Remarks	2-methyl-2-butene (CAS No. 513-35-9) also known as 2-methyl, 2-butene. The test substance was stable for the duration of all the studies performed by the test house.
Method	
Method/guideline followed	OECD Guide-line 201, EC Directive 92/69 C3, US EPA TSCA 797.1050 & 797.1060
GLP	Yes
Year	2003
Endpoint	Growth rate
Species	*Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) (Algae)
Exposure period	96 hours
Limit test	No
Analytical monitoring	Yes
Study Design	The study was conducted in completely filled (no headspace) and sealed vessels because of the volatility of 2-methyl-2-butene. The test media were prepared, either directly or by dilution, from an aqueous preparation in which the test substance was stirred in a sealed vessel for approximately 23 hours in the dark. After being allowed to stand for at least one hour to obtain an equilibrium concentration of 2-methyl-2-butene, aliquots of medium were removed from the middle of the vessel and after dilution and inoculated with algal cells, was used to fill the test vessels. The cultures were incubated in an orbital incubator under continuous illumination at temperatures ranging from 22.3 to 23.4°C for 96 hours. Replicate algal cultures, with an initial cell density of 1 x 10^4/ml, were exposed to 2-methyl-2-butene at nominal concentrations of 3.20, 7.04, 15.5, 34.1 and 75 mg/l. The exposure levels were monitored by measuring the concentrations of isoprene in samples of the test media using a GLC method of analysis. Cell densities were measured daily to monitor growth, and the test results are expressed in terms of the area under the growth curve and growth rate.
Evaluation of data	The area under the growth curve and the average specific growth rate are taken to be an index of growth and are calculated mathematically.

	The E_bC_{50} ("x" h) is the median effect concentration for inhibition of growth based on a comparison of areas under the growth curves after "x" hours. The E_bC_{50} was calculated using the moving average method of a computer program (Stephan:1977, 1982) which uses percentage effect and the nominal and measured test concentration in test samples. The E_rC_{50} ("x"- "y" h) is the median effect concentration for inhibition of growth based on a comparison of growth rates from "x" to "y" hours. The E_rC_{50} was calculated by either the moving average method or by non-linear interpolation between the two concentrations which bracket the 50% effect level of a computer program (Stephan:1977, 1982); the program uses percentage effect and the nominal and measured test concentration in test samples. The "no observed effect concentrations" (NOEC) was determined using Dunnett's multicomparison test to compare the percentage inhibition in the test group with that for the control cultures (Dunnett:1955, 1964).
Results	The measured concentrations of 2-methyl-2-butene ranged between 19 and 27% of their nominal values at the start of the test and between 22 and 29% of nominal after 96 hours. Based on an arithmetric mean, the overall mean measured levels of 2-methyl-2-butene were 0.689, 1.53, 3.61, 7.22 and 21.1 mg/l. Area under the growth curve (measured concentrations): E_bC_{50} (72 h) : 10.5 mg/l (95% confidence limits, 9.55 & 11.7 mg/l) E_bC_{50} (96 h) : 10.1 mg/l (95% confidence limits, 9.21 & 11.1 mg/l) No observed effect concentration (NOEC) : 3.61 mg/l Average specific growth rate (measured concentrations): E_rC_{50} (0-72 h) : 12.0 mg/l (95% confidence limits, 7.22 & 21.1 mg/l) E_rC_{50} (0-96 h) : 13.2 mg/l (95% confidence limits, 12.2 & 14.3 mg/l) No observed effect concentration (NOEC) : 7.22 mg/l
Observations	After 96 hours of exposure, the majority of the cells at 21.1 mg/l were swollen and/or mis-shapen.
Conclusions	After 72 and 96 hours of exposure to 2-methyl-2-butene, the E_bC_{50} values were 10.5 and 13.2 mg/l respectively; the E_rC_{50} values were 12.0 and 13.2 mg/l respectively. The "no observed effect concentration" (NOEC) for area under the growth curve and growth rate respectively, were 3.61 and 7.22 mg/l.
Data Quality ***Reference:***	Valid without restrictions Huntingdon Life Sciences Ltd. 2003. Algal growth inhibition assay. Project ID CSS 003 Huntingdon Life Sciences Ltd., Cambridgeshire, England
Other Last changed	21 January 2004 Robust summary prepared by contractor to Olefins Panel

ROBUST SUMMARY

Acute Toxicity to *Daphnia Magna*

Test Substance	
Remarks	2-methyl-2-butene (No. 513-35-9) The test substance was stable for the duration of all the studies performed by the test house.
Method	
Method/guideline followed	OECD Guide-line 202 and EC Directive 92/96 C2
GLP	Yes
Year	2002
Test type	Static
Species	*Daphnia magna* (Crustacea)
Exposure period	48 hours
Limit test	No
Analytical monitoring	Yes
Study Design	The study was conducted in completely filled (no headspace) and sealed vessels because of the volatility of 2-methyl-2-butene. The test media were prepared, either directly or by dilution, from an aqueous preparation in which the test substance was stirred in a sealed vessel in the dark for approximately 24 hours. After being allowed to stand for approximately 30 minutes to obtain an equilbrium concentration of 2-methyl-2-butene, aliquots of medium were removed from the middle of the vessel and used to fill replicate vessels at each concentration. Groups of twenty *Daphnia*, less than 24 hours old, were exposed for 48 hours to 2-methyl-2-butene, prepared in Elendt M4 medium at nominal concentrations of 2.13, 4.70, 10.3, 22.7 and 50.0 mg/l. The exposure levels were monitored by measuring the concentrations of 2-methyl-2-butene in samples of the test media using a GLC method of analysis. Observations of the *Daphnia* in each control and test vessel were made after 24 and 48 hours.
Evaluation of data	EC_{50} values were estimated either by the moving average method or by non-linear interpolation between the two concentrations which bracket the 50% effect level using a computer program (Stephan:1977;1982); the program uses the number of *Daphnia* exposed and the number immobile at each nominal and measured concentration. The "no observed effect concentration" (NOEC) was derived by direct inspection of the data on the immobility of the animals. An incidence of more than 10% is considered to be significant.

Results	Although lower than intended, the mean measured concentrations of 2-methyl-2-butene at the start (between 30 and 49% of their nominal values) were adequately maintained during the test, giving measured levels of between 28 and 46% of nominal after 48 hours. Based on an arithmetic mean, the overall mean measured levels of isoprene were 0.691, 1.74, 2.95, 6.63 and 23.6 mg/l. After 48 hours, the lowest measured concentration resulting in 100% immobility was 6.63 mg/l and the highest measured concentration at which immobilisation was ≤ 10% was 1.74 mg/l.
Conclusions	48-hour EC_{50} value = 3.84 mg/l (95% confidence limits of 3.01 and 4.80 mg/l; measured concentration) NOEC = 1.74 mg/l (measured concentration)
Data Quality	Valid without restrictions
Reference:	Huntingdon Life Sciences Ltd. 2002. Acute Toxicity to *Daphnia Magna*. Project ID CSS 033 Huntingdon Life Sciences Ltd., Cambridgeshire, England
Other	
Last changed	5 August 2003 Robust summary prepared by contractor to Olefins Panel

ROBUST SUMMARY

Acute Toxicity to Rainbow Trout (Semistatic conditions)

Test Substance	
Remarks	2-methyl-2-butene (CAS No. 513-35-9) also known as 2-methyl, 2-butene. The test substance was stable for the duration of all the studies performed by the test house.
Method	
Method/guideline followed	OECD Guide-line 203 and EC Directive 92/96 C1
GLP	Yes
Year	2002
Test type	Semistatic
Species	*Oncorhynchus mykiss* (Rainbow trout; fresh water fish)
Exposure period	96 hours
Limit test	No
Analytical monitoring	Yes
Study Design	The study was conducted in completely filled (no headspace) and sealed vessels because of the volatility of 2-methyl-2-butene. At each concentration, the test medium was prepared by stirring the test substance in a sealed vessel for approximately 24 hours. After being allowed to stand for at least 30 minutes to obtain an equilibrium concentration of 2-methyl-2-butene, aliquots of medium were removed from the middle of the vessel and used to fill the test vessels at each concentration. Groups of ten juvenile fish were exposed for 96 hours to a control solution (dechlorinated tap water, ca. 150-200 mg/l as $CaCO_3$) or to 2-methyl-2-butene at nominal concentrations of 2.13, 4.70, 10.3, 22.7 and 50.0 mg/l. The exposure levels were monitored by measuring the concentrations of 2-methyl-2-butene in samples of the test media using a GLC method of analysis. The measured concentrations of 2-methyl-2-butene ranged between 33% and 89% of their nominal values in samples of freshly prepared media and between 33 and 99% of their nominal values in samples of expired (24-hour-old) media (between 93 and 116% of their starting values). Based on an arithmetic mean, the overall mean measured levels of 2-methyl-2-butene were 1.67, 2.93, 5.33, 8.51 and 25.9 mg/l. Observations of the fish were made after 0.25, 2, 4, 24, 48, 72 and 96 hours of exposure.
Evaluation of data	LC_{50} values were estimated by non-linear interpolation between the two concentrations which bracket the 50% effect level using a computer program (Stephan:1977;1982); the program uses the number of fish exposed and the number dead at each measured concentration.

Results	After 96 hours, the highest measured concentration at which no mortality had occurred was 2.93 mg/l and the lowest at which there was 100% mortality was 8.51 mg/l. Treatment-related effects were exhibited at 5.33 mg/l and higher concentrations. Based on these findings the following values have been estimated: 96-hour LC_{50} value = 4.99 mg/l (95% confidence limits of 2.93 and 8.51 mg/l)
Conclusions	NOEC = 2.93 mg/l (measured concentration) LC_{50} = 4.99 mg/l (measured concentration)
Data Quality	Valid without restrictions
Reference:	Huntingdon Life Sciences Ltd. 2002. Acute Toxicity to Rainbow Trout (Semi-static exposure conditions). Project ID CSS 032. Huntingdon Life Sciences Ltd., Cambridgeshire, England
Other	
Last changed	21 January 2004 Robust summary prepared by contractor to Olefins Panel

ROBUST SUMMARY

Test Substance	
Remarks	2-methyl-2-butene (CAS No. 513-35-9). The test substance was stable for the duration of all studies performed at the test house. The carbon content of the test substance was determined before the start of the Closed Bottle test using a CEC Model 440 Elemental Analyser. The measured carbon content (85.47%) was equivalent to 99.8% of the theoretical value (85.63%), which was calculated using the empirical formula of 2-methyl-2-butene which comprised 98.2% of the test substance.
Method	
Method/guideline followed	OECD Guideline 301D and EC Directive 92/69, C.4-E and EPA OPPTS 835.3110.
GLP	Yes
Year	2001 - 2002
Type	Aerobic
Inoculum	Domestic sewage effluent
Concentration	2.1 mg/l related to test substance
Contact time	28 days
Test inoculum preparation	A sample of secondary effluent was collected on the day of the test from, a trickling-filter plant, which treats predominantly domestic waste. It was maintained under aerobic conditions in the laboratory, then, immediately before use, filtered through glass wool and the filtrate used as the inoculum for the test (1 ml filtrate/litre test medium).
Study design	Eighteen bottles were filled with a mineral salts medium, inoculated with unacclimated sewage effluent at a loading of 1ml/l, and the test substance at a nominal loading of 2.1 mg/l. The dissolved oxygen (DO) concentration in replicate bottles was measured on Days 0, 5, 7, 11, 14, 18, 21, 25 and 28. Four bottles were established for a concurrent five-day microbial inhibition assay, in which the biodegradation of the readily biodegradable reference substance, sodium benzoate, was examined in the presence of the test substance. DO concentration was measured in replicate bottles on Days 0 and 5. A further eighteen bottles were filled with mineral salts medium, inoculated with unacclimatised sewage effluent at a loading of 1 ml/l, and the reference substance, sodium benzoate at a nominal loading of 5 mg/l. DO concentrations in replicate bottles was measured on Days 0, 5, 7, 11, 14, 18, 21, 25 and 28. All test systems were incubated at 22 ± 2°C in darkness. Theoretical oxygen demands for the test and reference substances were based on their empirical formulae and molecular weights. The study was initiated on 8 October 2001.

Results	A maximum of 15% biodegradation was measured (on Day 25) by the end of the Closed Bottle test. The mean Total Viable Count of the sample of final sewage effluent in the main test was 1.0×10^5 Colony Forming Units (CFU) per ml and the mean count in inoculated MSM on Day 0 was 8.2×10^3 CFU/ml. The presence of the test substance was not considered to have caused any inhibitory affect on the normal degradative activity of the microbial inoculum on the reference substance in one replicate mixture containing both substances. There was discolouration of the contents of the second bottle of the inhibition assay, the pH was lower and there was no evidence of sodium benzoate degradation. Therefore, this result was considered to be erroneous.
Degradation Kinetic of test substance	7 (±) % after 28 day(s) 5 day(s) 2 and 4% 7 day(s) 1 and 2% 11 day(s) 0 and 1% 14 day(s) 2 and 2% 18 day(s) 4 and 4% 21 day(s) 2 and 6% 25 day(s) 12 and 15% 28 day(s) 4 and 10%
Control substance Kinetic	Benzoic acid, sodium salt 5 day(s) 67 and 68% 28 day(s) 83 and 85%
Conclusions ***Data Quality*** ***Reference***	Not biodegradable Valid without restriction Huntingdon Life Sciences Ltd. 2003. Assessment of biodegradability using the closed bottle method Project ID CSS 006. Huntingdon Life Sciences Ltd., Cambridgeshire, England.
Other Last changed	10 September 2003 Robust summary prepared by contractor to Olefins Panel

Robust Summary

Biodegradation

Test Substance:	2-Methyl, 2--Butene CAS No. 513-35-9 CAS Inventory Name: 2-Butene, 2-methyl-
Method/Guideline:	OECD Guideline 301F
Year (guideline):	1992
Type (test type):	Ready Biodegradability: Manometric Respirometry Test
GLP (Y/N):	Yes
Year (study performed):	2003
Inoculum:	Domestic activated sludge
Exposure Period:	28 Days
Test Conditions: • **Note: Concentration preparation, vessel type, replication, test conditions.**	Triplicate test systems were used to evaluate the biodegradability of the test and positive control substances at mean concentrations of 48/L and 46 mg/L, respectively. Blank test systems, which did not contain the test or positive control substance, were run concurrently in triplicate. The total suspended solids (TSS) of the activated sludge was determined to be 4.28 g/L. The inoculum was added at a 1% loading volume of sludge supernatant to test medium. The microbial count of the inoculum was 10^5 CFU/mL. One liter of test medium, which was aerated for 24 hours with carbon dioxide free air, was added to each one liter respirometer flask. The test substance was weighed in an air tight syringe and injected into the test medium. The test system was sealed immediately after addition of the test substance. An aliquot of the positive control stock solution was added to the appropriate test flasks. An unacclimated activated sludge inoculum was used in this study. The inoculum was obtained from the Clinton Sanitary Wastewater Treatment Plant, Annandale, NJ, USA. The treatment plant receives domestic sewage. All test systems were placed on a Coordinated Environmental Services (CES) automated respirometer which automatically recorded the oxygen uptake in general agreement with the OECD guideline. The 28-day study was conducted at a temperature range of 22 ± 1°C.

Results: **Units/Value:** **Note: Deviations from protocol or guideline analytical method.**	Biodegradation was based on oxygen consumption and the theoretical oxygen demand of the test substance as calculated using results of an elemental analysis of the test substance. By day 14, >60% biodegradation of positive control was observed, which meets the guideline requirement. No deviations from the protocol occurred that affected the integrity of the study data. No measurable biodegradation was observed in triplicate test systems therefore the test substance cannot be considered readily biodegradable. Sample — % Degradation* (day 28) — Mean % Degradation (day 28) Test Substance — 0, 0, 0 — 0 Na Benzoate — 91, 100, 89 — 93 * replicate data
Conclusion:	Not readily biodegradable
Reliability:	(1)-Reliable without restriction.
Reference:	ExxonMobil Biomedical Sciences, Inc. 2004. Ready Biodegradability: Manometric Respirometry test. Study # 183794A
Other (source):	Olefins Panel, American Chemistry Council

Repeated Dose Toxicity

Test Substance	
Remarks	2-methyl-2-butene (CAS number: 513-35-9) The test substance was stable for the duration of all studies performed at the test house.
Method	
Method/guideline followed	OECD 422
Test type	4 week general toxicity and reproduction/development toxicity screening test by inhalation exposure to rats (Toxicity phase)
GLP	Yes.
Year	2001/2002
Species	Rat
Strain	Crl:CD® (Sprague-Dawley) IGS BR
Route of administration	Inhalation (gas).
Duration of test	28 days
Doses/concentration levels	0, 580, 2000, or 7000 ppm
Sex	12 males, 12 females per dose group for main study group
Exposure period	6 hours/day.
Frequency of treatment	7 days/week
Control group and treatment	12 males, 12 females, air-only exposed.
Post exposure observation period	Not applicable.
Statistical methods	All statistical analyses were carried out separately for males and females. Data relating to food consumption was analysed on a cage basis. For all other parameters, the analyses were carried out using the individual animal as the basic experimental unit. The following data types were analysed at each timepoint separately:- Rearing and activity counts Bodyweight (FOB) and body temperature Grip strength, landing footsplay and motor activity. Bodyweight, using gains over appropriate study periods. Food consumption, over appropriate study periods, using cumulative cage totals. Blood chemistry and haematology Organ weights, absolute and/or adjusted for terminal bodyweight Pathological findings, for the number of animals with and without each finding. For categorical data, including rearing and activity counts and pathological findings, the proportion of animals was analysed using Fisher's Exact test (Fisher 1973) for each treated group versus the control.

	For continuous data, Bartlett's test (Bartlett 1937) was first applied to test the homogeneity of variance between the groups. Using tests dependent on the outcome of Bartlett's test, treated groups were then compared with the Control group, incorporating adjustment for multiple comparisons where necessary. The following sequence of statistical tests was used for bodyweight (FOB), body temperature, grip strength, landing foot splay and motor activity, bodyweight, food consumption, organ weight and clinical pathology data. If 75% of the data (across all groups) were the same value, for example c, then a frequency analysis was applied. Treatment groups were compared using a Mantel test for a trend in proportions (Mantel 1963) and also pairwise Fisher's Exact tests (Fisher 1973) for each dose group against the control both for i) values <c versus values >=c, and for ii) values <=c versus values >c, as applicable. If Bartlett's test for variance homogeneity (Bartlett 1937) was not significant at the 1% level, then parametric analysis was applied. If the F1 test for monotonicity of dose-response (Healey 1999) was not significant at the 1% level, Williams' test for a monotonic trend (Williams 1971, 1972) was applied. If the F1 test was significant, suggesting that the dose-response was not monotone, Dunnett's test (Dunnett 1955, 1964) was performed instead. If Bartlett's test was significant at the 1% level, then logarithmic and square-root transformations were tried. If Bartlett's test was still significant, then non-parametric tests were applied. If the H1 test for monotonicity of dose-response (Healey 1999) was not significant at the 1% level, Shirley's test for a monotonic trend (Shirley 1977) was applied. If the H1 test was significant, suggesting that the dose-response was not monotone, Steel's test (Steel 1959) was performed instead. For organ weight data, analysis of variance was initially performed using terminal bodyweight as covariate. If the within group relationship between organ weight and bodyweight was significant at the 10% level (Angervall and Carlstrom, 1963), then the treatment comparisons were made on adjusted group means in order to allow for differences in bodyweight which might influence the organ weights. Significant differences between Control and treated groups were expressed at the 5% ($p<0.05$) or 1% ($p<0.01$) level. Williams test is denoted by '*'; t tests are denoted by '+', Dunnett's test is denoted by '$\underline{*}$' and Shirley's test by '$\underline{+}$'.

Test Conditions	Groups of 12 male and 12 female CD rats were exposed to the test material as a gas daily by inhalation for six hours/day at exposure levels of 0, 580, 2000, or 7000 ppm. In this main study (repeated-exposure general toxicity) males and females were exposed for 28 days. During the study, clinical condition, detailed functional observational battery, motor activity, bodyweight, food consumption, haematology, blood chemistry, organ weight and macroscopic and microscopic pathology investigations were undertaken. The study also contained reproductive/developmental toxicity satellite groups (summarized separately).
Results	
NOAEL (NOEL)	580 ppm .
LOAEL (LOEL)	Not applicable.
Remarks	The test atmospheres were analysed by GC and the analysed concentrations were in agreement with the target concentrations. Toxicity Phase Clinical signs during exposure included half-closed eyes on Day 1 at 2000 and 7000 ppm, and a lower level of response to external stimuli. This latter finding also occurred on one further occasion at 7000 ppm. There were no signs considered reflective of a general systemic effect observed during routine clinical examination or during the functional observational battery. There was a slightly lower bodyweight gain at 7000 ppm, and slightly longer clotting times at 2000 ppm (prothrombin time for females) and 7000 ppm (prothrombin times for both sexes and activated partial thromboplastin time for males). Cholesterol levels were increased amongst females exposed to 7000 ppm but in the absence of any further effects in the clinical chemistry parameters or the males this is of uncertain significance. Pathological changes were noted amongst high dose females in the liver, evidenced as an increased organ weight and minimal centrilobular hepatocyte hypertrophy. There was a decreased incidence of extramedullary haemopoiesis of the spleen of high dose animals, an increase in goblet cell hyperplasia in the nasal passages of high dose males, and, amongst high and intermediate dose males, a slight increase in severity of mycardial inflammatory heart lesions and cortical/medullary tubular basophila in the kidneys.

Conclusions	Slight effects on general systemic toxicity due to the test substance were apparent amongst animals receiving 7000 ppm, and to a lesser extent at 2000 ppm. The no effect level of the test substance for the general systemic toxicity to rats for 28 days inhalation administration was 580 ppm.
Data Quality	
Reliabilities	Klimisch value = 1 (Reliable without restrictions).
References	Huntingdon Life Sciences Ltd., 2004 4-week general toxicity and reproduction/development toxicity screening test by inhalation exposure to rats Project ID CSS 002. Huntingdon Life Sciences Ltd., Cambridgeshire, England.
Other	
Last changed	11 September 2003 Robust summary prepared by contract to Olefins Panel

Toxicity to Reproduction

Test Substance	
Remarks	2-methyl-2-butene (CAS number: 513-35-9) The test substance was stable for the duration of all studies performed at the test house.
Method	
Method/guideline followed	OECD 422
Test type	4 week general toxicity and reproduction/development toxicity screening test by inhalation exposure to rats (Reproductive phase)
GLP	Yes.
Year	2001/2002
Species	Rat
Strain	Crl:CD® (Sprague-Dawley) IGS BR
Route of administration	Inhalation (gas).
Duration of test	Two weeks prior to breeding, during breeding, and continuing through day 19 of gestation. The dams were then allowed to deliver their litters, which were retained until lactation day 4.
Doses/concentration levels	0, 580, 2000, or 7000 ppm
Sex	12 females per dose group for this satellite study.
Exposure period	6 hours/day.
Frequency of treatment	7 days/week
Control group and treatment	12 females, air-only exposed.
Post exposure observation period	Not applicable.
Statistical methods	All statistical analyses were carried out separately for males and females. Data relating to food consumption was analysed on a cage basis. For all other parameters, the analyses were carried out using the individual animal as the basic experimental unit. The following data types were analysed at each timepoint separately:- Rearing and activity counts Bodyweight (FOB) and body temperature Grip strength, landing footsplay and motor activity. Bodyweight, using gains over appropriate study periods. Food consumption, over appropriate study periods, using cumulative cage totals. Blood chemistry and haematology Organ weights, absolute and/or adjusted for terminal bodyweight Pathological findings, for the number of animals with and without each finding. For categorical data, including rearing and activity counts and pathological findings, the proportion of animals was analysed using Fisher's Exact test (Fisher 1973) for each treated group versus the control.

	For continuous data, Bartlett's test (Bartlett 1937) was first applied to test the homogeneity of variance between the groups. Using tests dependent on the outcome of Bartlett's test, treated groups were then compared with the Control group, incorporating adjustment for multiple comparisons where necessary. The following sequence of statistical tests was used for bodyweight (FOB), body temperature, grip strength, landing foot splay and motor activity, bodyweight, food consumption, organ weight and clinical pathology data. If 75% of the data (across all groups) were the same value, for example c, then a frequency analysis was applied. Treatment groups were compared using a Mantel test for a trend in proportions (Mantel 1963) and also pairwise Fisher's Exact tests (Fisher 1973) for each dose group against the control both for i) values <c versus values >=c, and for ii) values <=c versus values >c, as applicable. If Bartlett's test for variance homogeneity (Bartlett 1937) was not significant at the 1% level, then parametric analysis was applied. If the F1 test for monotonicity of dose-response (Healey 1999) was not significant at the 1% level, Williams' test for a monotonic trend (Williams 1971, 1972) was applied. If the F1 test was significant, suggesting that the dose-response was not monotone, Dunnett's test (Dunnett 1955, 1964) was performed instead. If Bartlett's test was significant at the 1% level, then logarithmic and square-root transformations were tried. If Bartlett's test was still significant, then non-parametric tests were applied. If the H1 test for monotonicity of dose-response (Healey 1999) was not significant at the 1% level, Shirley's test for a monotonic trend (Shirley 1977) was applied. If the H1 test was significant, suggesting that the dose-response was not monotone, Steel's test (Steel 1959) was performed instead. For organ weight data, analysis of variance was initially performed using terminal bodyweight as covariate. If the within group relationship between organ weight and bodyweight was significant at the 10% level (Angervall and Carlstrom, 1963), then the treatment comparisons were made on adjusted group means in order to allow for differences in bodyweight which might influence the organ weights. Significant differences between Control and treated groups were expressed at the 5% ($p<0.05$) or 1% ($p<0.01$) level. Williams test is denoted by '*'; t tests are denoted by '+', Dunnett's test is denoted by '$\underline{*}$' and Shirley's test by '$\underline{+}$'.

Test Conditions	Satellite groups of 12 female Sprague Dawley rats were exposed to the test material as a gas daily by inhalation for six hours/day at exposure levels of 0, 580, 2000, or 7000 ppm. The study design included a main study for repeated dose toxicity end points (summarized separately) and reproductive / developmental toxicity satellite groups of 12 females per exposure level. The reproductive/developmental toxicity satellite groups were exposed for two weeks prior to breeding, during breeding and continuing through day 19 of gestation. Males from the main study were used to breed these females. The dams were allowed to deliver their litters, which were retained until lactation Day 4. During the study clinical condition, bodyweight, food consumption, oestrus cycles, mating performance, litter data, organ weights and macroscopic pathology were undertaken.
Results	
NOAEL (NOEL)	7000 ppm.
LOAEL (LOEL)	Not applicable.
Remarks	The test atmospheres were analysed by GC and the analysed concentrations were in agreement with the target concentrations. Reproductive Phase Exposure to female rats for 2 weeks prior to pairing, and up to Day 19 of gestation, did not produce any evidence of any reproduction or developmental toxicity. The oestrus cycle was unaffected by exposure, and mating performance, fertility indices and gestation length were similar in all groups. There were no adverse effects upon survival or growth of the offspring in utero or up to Day 4 of lactation.
Conclusions	The no effect level for reproduction/developmental toxicity was 7000 ppm
Data Quality	
Reliabilities	Klimisch value = 1 (Reliable without restrictions).
References	Huntingdon Life Sciences Ltd., 2004 4-week general toxicity and reproduction/development toxicity screening test by inhalation exposure to rats Project ID CSS 002. Huntingdon Life Sciences Ltd., Cambridgeshire, England.
Other	
Last changed	11 September 2003 Robust summary prepared by contractor to Olefins Panel

APPENDIX 7

ROBUST SUMMARY

Algal growth inhibition assay

Test Substance	
Remarks	Pyrolysis C5s (CAS No. 68476-55-1, hydrocarbons, C5-rich) are produced as a C5 hydrocarbons distillate fraction from pyrolysis gasoline obtained from ethylene production (steam cracking process). The sample tested consisted of isoprene (18%), cis- and trans-pentadiene-1,3 (16%), cyclopentadiene + dicyclopentadiene (14%), n-pentane (10%), cyclopentene (7%), 2-methyl-2-butene (3%) and cyclopentane (1%). The balance consists of other hydrocarbons with similar boiling points (primarily C5s). Alternative CAS numbers for the test substance are, 68476-43-7, 68527-19-5, 68603-00-9 and 68956-55-8. The three major components analysed were shown to be stable for the duration of testing in the study.
Method	
Method/guideline followed	OECD Guide-line 201, EC Directive 92/69 C3, US EPA TSCA 797.1050 & 797.1060
GLP	Yes
Year	2002
Endpoint	Growth rate
Species	*Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) (Algae)
Exposure period	96 hours
Limit test	No
Analytical monitoring	Yes
Study design	The study was conducted in completely filled (no headspace) and sealed vessels because of the volatility of Pyrolysis C5s. The test medium was prepared by stirring the test substance in a sealed mixing vessel for approximately 21 hours in the dark. After being allowed to stand for approximately 90 minutes to obtain an equilibrium concentration of the test substance, a portion of the medium was removed from the middle of the vessel and after the inoculation with algal cells, the medium was used to fill replicate test vessels at each concentration. The cultures were incubated in an orbital incubator under continuous illumination at temperatures ranging from 23.1 to 24.4°C for 96 hours. Replicate algal cultures, with an initial cell density of 1 x 10^4/ml, were exposed to Pyrolysis C5s at nominal concentrations of 4.27, 9.39, 20.7, 45.5 and 100 mg/l.

Study Design	The exposure levels were monitored by measuring the concentrations of Pyrolysis C5s in samples of the test media using an HPLC method of analysis. Cell densities were measured daily to monitor growth, and the test results are expressed in terms of the area under the growth curve and growth rate.
Evaluation of data	The area under the growth curve and the average specific growth rate are taken to be an index of growth and are calculated mathematically. The E_bL_{50} /E_bC_{50} ("x" h) is the median effect concentration for inhibition of growth based on a comparison of areas under the growth curves after "x" hours. The E_bL_{50} /E_bC_{50} was calculated by the moving average method using a computer program (Stephan:1977, 1982); the program uses percentage effect and the nominal loading rates and measured test concentration in test samples. The E_rL_{50} /E_rC_{50} ("x"- "y" h) is the median effect loading rate / concentration for inhibition of growth based on a comparison of growth rates from "x" to "y" hours. The E_rL_{50} /E_rC_{50} was calculated by the moving average method using a computer program (Stephan:1977, 1982); the program uses percentage effect and the nominal loading rate and measured test concentration in test samples. The "no observed effect loading rate/ concentration" (NOELR/NOEC) was determined using Dunnett's multicomparison test to compare the percentage inhibition in the test group with that for the control cultures (Dunnett:1955, 1964).

Results	The measured concentrations of Pyrolysis C5s ranged between 15 and 32% of their nominal values at the start of the test and between 20 and 38% of nominal after 96 hours. Based on an arithmetric mean, the overall mean measured levels of Pyrolysis C5s were 1.22, 3.26, 6.47, 7.84 and 30.6 mg/l. Area under the growth curve: E_bL_{50} (72 h) : 58.1 mg/l (95% confidence limits, 53.7 & 62.3 mg/l) E_bL_{50} (96 h) : 56.0 mg/l (95% confidence limits, 51.7 & 60.0 mg/l) E_bC_{50} (72 h) : 12.4 mg/l (95% confidence limits, 11.3 & 13.6 mg/l) E_bC_{50} (96 h) : 11.7 mg/l (95% confidence limits, 10.8 & 12.8 mg/l) No observed effect loading rate (NOELR) : 9.39 mg/l No observed effect concentratiopn (NOEC) : 3.26 mg/l. Average specific growth rate (measured concentrations): E_rL_{50} (72 h) : 75.6 mg/l (95% confidence limits, 70.4 & 81.9 mg/l) E_rL_{50} (96 h) : 74.4 mg/l (95% confidence limits, 69.6 & 80.0 mg/l) E_rC_{50} (72 h) : 18.9 mg/l (95% confidence limits, 16.7 & 21.7 mg/l) E_rC_{50} (96 h) : 18.4 mg/l (95% confidence limits, 16.4 & 20.8 mg/l) No observed effect loading rate (NOELR) : 45.5 mg/l No observed effect concentration (NOEC) : 7.84 mg/l.
Observations	After 96 hours of exposure, the cells at 100 mg/l (30.6 mg/l, measured) were swollen and/or mis-shapen.
Conclusions	After 96 hours of exposure to Pyrolysis C5s, the E_bL_{50}/E_bC_{50} values were 56.0 and 11.7 mg/l respectively; the E_rL_{50}/E_rC_{50} values were 74.4 and 18.4 mg/l respectively. The "no observed effect loading rate / concentration" (NOELR/NOEC) for area under the growth curve respectively were 9.39 and 3.26 mg/l. For growth rate, the NOELR/NOEC respectively were 45.5 and 7.84 mg/l.
Data Quality	Valid without restrictions
Reference:	Huntingdon Life Sciences Ltd. 2002. Algal growth inhibition assay. Project ID CSS 013 Huntingdon Life Sciences Ltd., Cambridgeshire, England
Other	
Last changed	24 May 2004 Robust summary prepared by contractor to Olefins Panel

APPENDIX 7

ROBUST SUMMARY

Acute toxicity to *Daphnia magna*

Test Substance	
Remarks	Pyrolysis C5s (CAS No. 68476-55-1, hydrocarbons, C5-rich) are produced as a C5 hydrocarbons distillate fraction from pyrolysis gasoline obtained from ethylene production (steam cracking process). The sample tested consisted of isoprene (18%), cis- and trans-pentadiene-1,3 (16%), cyclopentadiene + dicyclopentadiene (14%), n-pentane (10%), cyclopentene (7%), 2-methyl-2-butene (3%) and cyclopentane (1%). The balance consists of other hydrocarbons with similar boiling points (primarily C5s). Alternative CAS numbers for the test substance are, 68476-43-7, 68527-19-5, 68603-00-9 and 68956-55-8. The three major components analysed were shown to be stable for the duration of testing in the study.
Method	
Method/guideline followed	OECD Guide-line 202 and EC Directive 92/96 C2
GLP	Yes
Year	2002-2003
Test type	Static
Species	*Daphnia magna* (Crustacea)
Exposure period	48 hours
Limit test	No
Analytical monitoring	Yes
Study Design	The study was conducted in completely filled (no headspace) and sealed vessels because of the volatility of Pyrolysis C5s. At each concentration, the test medium was prepared from an aqueous preparation in which the test substance was stirred in a sealed vessel in the dark for approximately 23 hours. After being allowed to stand for approximately 1.5 hours to obtain an equilbrium concentration of Pyrolysis C5s, aliquots of medium were removed from the middle of the vessel and used to fill replicate vessels at each concentration. Groups of twenty *Daphnia*, less than 24 hours old, were exposed for 48 hours to Pyrolysis C5s, prepared in Elendt M4 medium at nominal concentrations of 2.13, 4.70, 10.3, 22.7 and 50.0 mg/l. The exposure levels were monitored by measuring the concentrations of Pyrolysis C5s in samples of the test media using an HPLC method of analysis. Observations of the *Daphnia* in each control and test vessel were made after 24 and 48 hours.

Evaluation of data	EL_{50} / EC_{50} values were estimated by non-linear interpolation between the two loading rates / concentrations which bracket the 50% effect level using a computer program (Stephan:1977;1982); the program uses the number of *Daphnia* exposed and the number immobile at each nominal loading rate and measured concentration.
Results	Although lower than intended, the mean measured concentrations of Pyrolysis C5s at the start (between 54 and 67% of their nominal values) were adequately maintained during the test, giving measured levels of between 55 and 73% of nominal after 48 hours. Based on a arithmetic mean, the overall mean measured levels of Pyrolysis C5s were 1.41, 3.23, 6.83, 15.6 and 27.2 mg/l. After 48 hours, the highest loading rate at which no immobilisation occurred was 4.70 mg/l and the lowest loading rate resulting in 100% immobility was 10.3 mg/l; 3.23 and 6.83 mg/l in terms of mean measured Pyrolysis C5s concentrations. 48-hour EL_{50} value = 6.96 mg/l (95% confidence limits of 4.70 and 10.3 mg/l; nominal loading rate) 48-hour EC_{50} value = 4.70 mg/l (95% confidence limits of 3.23 and 6.83 mg/l; measured concentration)
Conclusions	48-hour EL_{50} = 6.96 mg/l (nominal loading rate) 48-hour EC_{50} = 4.70 mg/l (measured concentration)
Data Quality	Valid without restrictions
Reference:	Huntingdon Life Sciences Ltd. 2002. Acute Toxicity to *Daphnia Magna*. Project ID CSS 014 Huntingdon Life Sciences Ltd., Cambridgeshire, England
Other Last changed	21 July 2004 Robust summary prepared by contractor to Olefins Panel

APPENDIX 7

ROBUST SUMARY

Acute Toxicity to Rainbow Trout (Semi-static conditions)

Test Substance	
Remarks	Pyrolysis C5s (CAS No. 68476-55-1, hydrocarbons, C5-rich) are produced as a C5 hydrocarbons distillate fraction from pyrolysis gasoline obtained from ethylene production (steam cracking process). The sample tested consisted of isoprene (18%), cis- and trans-pentadiene-1,3 (16%), cyclopentadiene + dicyclopentadiene (14%), n-pentane (10%), cyclopentene (7%), 2-methyl-2-butene (3%) and cyclopentane (1%). The balance consists of other hydrocarbons with similar boiling points (primarily C5s). Alternative CAS numbers for the test substance are, 68476-43-7, 68527-19-5, 68603-00-9 and 68956-55-8. The three major components analysed were shown to be stable for the duration of testing in the study.
Method	
Method/guideline followed	OECD Guide-line 203 and EC Directive 92/96 C1
GLP	Yes
Year	2002
Test type	Semistatic
Species	*Oncorhynchus mykiss* (Rainbow trout; fresh water fish)
Exposure period	96 hours
Limit test	No
Analytical monitoring	Yes
Study Design	The study was conducted in completely filled (no headspace) and sealed vessels because of the volatility of Pyrolysis C5s. At each concentration, the test medium was prepared by stirring the test substance in a sealed vessel for between approximately 21 and 22 hours. After being allowed to stand for at least 60 minutes to obtain an equilibrium concentration of Pyrolysis C5s, aliquots of medium were removed from the middle of the vessel and used to fill the test vessels at each concentration. Groups of ten juvenile fish were exposed for 96 hours to a control solution (dechlorinated tap water, ca. 150-200 mg/l as $CaCO_3$) or to Pyrolysis C5s at nominal concentrations of 2.13, 4.70, 10.3, 22.7 and 50.0 mg/l. The exposure levels were monitored by measuring the concentrations of Pyrolysis C5s in samples of the test media using a HPLC method of analysis. Observations of the fish were made after 0.25, 2, 4, 24, 48, 72 and 96 hours of exposure.

Evaluation of data	LL_{50}/LC_{50} values were estimated by non-linear interpolation between the two loading rates / concentrations which bracket the 50% effect level using a computer program (Stephan:1977;1982); the program uses the number of fish exposed and the number dead at each nominal loading rate / measured concentration.
Results	The measured concentrations of Pyrolysis C5s ranged between 37% and 91% of their nominal values in samples of freshly prepared media and between 24 and 73% of their nominal values in samples of expired (24-hour-old) media (between 62 and 112% of their starting values). Based on an arithmetic mean, the overall mean measured levels of Pyrolysis C5s were 1.42, 2.49, 6.40, 12.8 and 27.0 mg/l. After 96 hours, the highest nominal loading rate at which ≤10% mortality had occurred was 10.3 mg/l and the lowest at which there was 100% mortality was 22.7 mg/l; 6.40 and 12.8 mg/l in terms of mean measured Pyrolysis C5s concentrations. Treatment-related effects were exhibited at nominal loading rates of 4.70 mg/l and higher rates (2.49 mg/l, measured). Based on these findings the following values have been estimated: 96-hour LL_{50} value = 14.1 mg/l (95% confidence limits of 10.3 and 22.7 mg/l). 96-hour LC_{50} value = 8.41 mg/l (95% confidence limits of 6.40 and 12.8 mg/l).
Conclusions	96-hour LL_{50} = 14.1 mg/l (loading rate) 96-hour LC_{50} = 8.41 mg/l (measured concentration)
Data Quality	Valid without restrictions
Reference:	Huntingdon Life Sciences Ltd. 2002. Acute Toxicity to Rainbow Trout (Semi-static exposure conditions). Project ID CSS 015. Huntingdon Life Sciences Ltd., Cambridgeshire, England
Other Last changed	1 June 2004 Robust summary prepared by contractor to Olefins Panel

APPENDIX 9

ROBUST SUMMARY

Test Substance	
Remarks	Pyrolysis C5s (CAS No. 68476-55-1, hydrocarbons, C5-rich) are produced from C5 hydrocarbons distillate fraction from pyrolysis gasoline obtained from ethylene production (steam cracking process). The sample tested consisted of isoprene (18%), cis- and trans-pentadiene-1,3 (16%), cyclopentadiene + dicyclopentadiene (14%), n-pentane (10%), cyclopentene (7%), 2-methyl-2-butene (3%) and cyclopentane (1%). The balance consists of other hydrocarbons with similar boiling point (primarily C5s). Alternative CAS numbers for the test substance are, 68476-43-7, 68527-19-5, 68603-00-9 and 68956-55-8. The three major components analysed were shown to be stable for the duration of testing in this study. The carbon content of the test substance was determined before the start of the Closed Bottle test using a CEC Model 440 Elemental Analyser. The measured carbon content (88%) was equivalent to 102% of the theoretical value (85.9%), which was based on the pre-shipment composition of the test substance.
Method	
Method/guideline followed	OECD Guideline 301D and EC Directive 92/69, C.4-E and EPA OPPTS 835.3110.
GLP	Yes
Year	2002
Type	Aerobic
Inoculum	Domestic sewage effluent
Concentration	2 mg/l related to test substance
Contact time	28 days
Test inoculum preparation	A sample of secondary effluent was collected on the day of the test from, a trickling-filter plant, which treats predominantly domestic waste. It was maintained under aerobic conditions in the laboratory, then, immediately before use, filtered through glass wool and the filtrate used as the inoculum for the test (1 ml filtrate/litre test medium).
Study design	Eighteen bottles were filled with a mineral salts medium, inoculated with unacclimated sewage effluent at a loading of 1 ml/l, and the test substance at a nominal loading of 2 mg/l. The dissolved oxygen (DO) concentration in replicate bottles was measured on Days 0, 5, 7, 11, 14, 18, 21, 25 and 28. Four bottles were established for a concurrent five-day microbial inhibition assay, in which the biodegradation of the readily biodegradable reference substance, sodium benzoate, was examined in the presence of the test substance. DO concentration was measured in replicate bottles on Days 0 and 5. A further eighteen bottles were filled with mineral salts medium, inoculated with unacclimatised sewage effluent at a loading of 1 ml/l, and the reference substance, sodium benzoate at a nominal loading of 5 mg/l. DO concentrations in replicate bottles was measured on Days 0, 5, 7, 11, 14, 18, 21, 25 and 28.

	All test systems were incubated at 22 ± 2°C in darkness. Theoretical oxygen demands for the test and reference substances were based on calculated empirical formulae and molecular weights. The study was initiated on 13 March 2002.
Results	A maximum of 9% biodegradation was measured (on Day 28) by the end of the Closed Bottle test. The mean Total Viable Count of the sample of final sewage effluent in the main test was 1.9 x 10^5 Colony Forming Units (CFU) per ml and the mean count in inoculated MSM on Day 0 was 1.4 x 10^4 CFU/ml. The presence of the test substance was not considered to have caused any inhibitory affect on the normal degradative activity of the microbial inoculum on the reference substance.
Degradation Kinetic of test substance	8 (±) % after 28 day(s) 5 day(s) 3 and 3% 7 day(s) 4 and 6% 11 day(s) 0 and 2% 14 day(s) 0 and 0% 18 day(s) 8 and 8% 21 day(s) 1 and 3% 25 day(s) 4 and 6% 28 day(s) 5 and 9%
Control substance Kinetic	Benzoic acid, sodium salt 5 day(s) 69 and 70% 28 day(s) 81 and 83%
Conclusions ***Data Quality*** ***Reference***	Not biodegradable Valid without restriction Huntingdon Life Sciences Ltd. 2002. Assessment of biodegradability using the closed bottle method Project ID CSS 016. Huntingdon Life Sciences Ltd., Cambridgeshire, England.
OtherLast changed	21 July 2004 Robust summary prepared by contractor to Olefins Panel

Robust Summary

Biodegradation

Test Substance:	Pyrolysis C5s CAS Number — CAS Inventory Name 68476-55-1[1] — Hydrocarbons, C5-rich 68476-43-7 — Hydrocarbons, C4-6, C5-rich 68527-19-5 — Hydrocarbons, C1-4, debutanizer fraction 68603-00-9 — Distillates, petroleum, thermal cracked naphtha and gas oil 68956-55-8 — Hydrocarbons, C5-unsatd. Note 1: This CAS number has been designated as the suggested CAS number for use in identifying the industry stream in future reporting. Other CAS numbers shown in the list above have been used to identify the stream, and may also be used in the future.
Method/Guideline:	OECD Guideline 301F
Year (guideline):	1992
Type (test type):	Ready Biodegradability: Manometric Respirometry Test
GLP (Y/N):	Yes
Year (study performed):	2003
Inoculum:	Domestic activated sludge
Exposure Period:	28 Days
Test Conditions: • **Note: Concentration preparation, vessel type, replication, test conditions.**	Triplicate test systems were used to evaluate the biodegradability of the test and positive control substances at mean concentrations of 49/L and 46 mg/L, respectively. Blank test systems, which did not contain the test or positive control substance, were run concurrently in triplicate. The total suspended solids (TSS) of the activated sludge was determined to be 4.28 g/L. The inoculum was added at a 1% loading volume of sludge supernatant to test medium. The microbial count of the inoculum was 10^5 CFU/mL. One liter of test medium, which was aerated for 24 hours with carbon dioxide free air, was added to each one liter respirometer flask. The test substance was weighed in an air tight syringe and injected into the test medium. The test system was sealed immediately after addition of the test substance. An aliquot of the positive control stock solution was added to the appropriate test flasks. An unacclimated activated sludge inoculum was used in this study. The inoculum was obtained from the Clinton Sanitary Wastewater Treatment Plant, Annandale, NJ, USA. The treatment plant receives domestic sewage. All test systems were placed on a Coordinated Environmental Services (CES) automated respirometer which automatically recorded the oxygen uptake in general agreement with the OECD guideline. The 28-day study was conducted at a temperature range of 22 ± 1°C.

Results: **Units/Value:** **Note: Deviations from protocol or guideline analytical method.**	Biodegradation was based on oxygen consumption and the theoretical oxygen demand of the test substance as calculated using results of an elemental analysis of the test substance. By day 14, >60% biodegradation of positive control was observed, which meets the guideline requirement. No deviations from the protocol occurred that affected the integrity of the study data. No measurable biodegradation was observed in triplicate test systems therefore the test substance cannot be considered readily biodegradable. Sample — % Degradation* (day 28) — Mean % Degradation (day 28) Test Substance — 0, 0, 0 — 0 Na Benzoate — 91, 100, 89 — 94 * replicate data
Conclusion:	Not readily biodegradable
Reliability:	(1)-Reliable without restriction.
Reference:	ExxonMobil Biomedical Sciences, Inc. 2004. Ready Biodegradability: Manometric Respirometry test. Study # 183994A
Other (source):	Olefins Panel, American Chemistry Council

APPENDIX 5

Robust summary

PHYSICOCHEMICAL PROPERTIES

Test Substance	
Remarks	Pyrolysis C5s (CAS No. 68476-55-1, hydrocarbons, C5-rich) are produced as a C5 hydrocarbons distillate fraction from pyrolysis gasoline obtained from ethylene production (steam cracking process). The sample tested consisted of isoprene (18%), cis- and trans-pentadiene-1,3 (16%), cyclopentadiene + dicyclopentadiene (14%), n-pentane (10%), cyclopentene (7%), 2-methyl-2-butene (3%) and cyclopentane (1%). The balance consists of other hydrocarbons with similar boiling points (primarily C5s). Alternative CAS numbers for the test substance are 68476-43-7, 68527-19-5, 68603-00-9 and 68956-55-8. The three major components analysed were shown to be stable for the duration of the study.
Method	
Method/guideline followed	OECD Method 103
Test type	Boiling temperature
GLP	Yes.
Year	2002
Results	25.0°C to 56.5°C
Boiling point	Main components at 37.0°C and 47.0°C
Remarks	The test substance has two main boiling temperatures of 36.8°C and 46.9° where a continuous string of bubbles is observed. Apart from these temperatures, between 24.8°C and 56.5°C a slow release of bubbles (one at a time) was observed coming from the tube.
Data Quality	Klimish value = 1 (Reliable without restrictions)
Reference:	Huntingdon Life Sciences Ltd. 2002. Physicochemical properties. Project ID CSS035. Huntingdon Life Sciences Ltd., Cambridgeshire, England.
Other	
Last changed	8 June 2004 Robust summary prepared by contractor to Olefins Panel

APPENDIX 5 (continued)

PHYSICOCHEMICAL PROPERTIES

Test Substance	
Remarks	Pyrolysis C5s (CAS No. 68476-55-1, hydrocarbons, C5-rich) are produced as a C5 hydrocarbons distillate fraction from pyrolysis gasoline obtained from ethylene production (steam cracking process). The sample tested consisted of isoprene (18%), cis- and trans-pentadiene-1,3 (16%), cyclopentadiene + dicyclopentadiene (14%), n-pentane (10%), cyclopentene (7%), 2-methyl-2-butene (3%) and cyclopentane (1%). The balance consists of other hydrocarbons with similar boiling points (primarily C5s). Alternative CAS numbers for the test substance are 68476-43-7, 68527-19-5, 68603-00-9 and 68956-55-8. The three major components analysed were shown to be stable for the duration of the study.
Method	
Method/guideline followed	OECD Method 104
Test type	Vapour Pressure
GLP	Yes.
Year	2002
Results	
Vapour pressure	58484 Pa at 25°C
Remarks	
Data Quality	Klimish value = 1 (Reliable without restrictions)
Reference:	Huntingdon Life Sciences Ltd. 2002. Physicochemical properties. Project ID CSS035. Huntingdon Life Sciences Ltd., Cambridgeshire, England.
Other	
Last changed	8 June 2004 Robust summary prepared by contractor to Olefins Panel

APPENDIX 5 (continued)

PHYSICOCHEMICAL PROPERTIES

Test Substance	
Remarks	Pyrolysis C5s (CAS No. 68476-55-1, hydrocarbons, C5-rich) are produced as a C5 hydrocarbons distillate fraction from pyrolysis gasoline obtained from ethylene production (steam cracking process). The sample tested consisted of isoprene (18%), cis- and trans-pentadiene-1,3 (16%), cyclopentadiene + dicyclopentadiene (14%), n-pentane (10%), cyclopentene (7%), 2-methyl-2-butene (3%) and cyclopentane (1%). The balance consists of other hydrocarbons with similar boiling points (primarily C5s). Alternative CAS numbers for the test substance are 68476-43-7, 68527-19-5, 68603-00-9 and 68956-55-8. The three major components analysed were shown to be stable for the duration of the study.
Method	
Method/guideline followed	OECD Method 105 (Flask shake method)
Test type	Water solubility
GLP	Yes.
Year	2002
Results	
Water solubility	0.72 g/l at 20°C
Remarks	
Data Quality	Klimish value = 1 (Reliable without restrictions)
Reference:	Huntingdon Life Sciences Ltd. 2002. Physicochemical properties. Project ID CSS035. Huntingdon Life Sciences Ltd., Cambridgeshire, England.
Other	
Last changed	8 June 2004 Robust summary prepared by contractor to Olefins Panel

APPENDIX 5 (continued)

PHYSICOCHEMICAL PROPERTIES

Test Substance	
Remarks	Pyrolysis C5s (CAS No. 68476-55-1, hydrocarbons, C5-rich) are produced as a C5 hydrocarbons distillate fraction from pyrolysis gasoline obtained from ethylene production (steam cracking process). The sample tested consisted of isoprene (18%), cis- and trans-pentadiene-1,3 (16%), cyclopentadiene + dicyclopentadiene (14%), n-pentane (10%), cyclopentene (7%), 2-methyl-2-butene (3%) and cyclopentane (1%). The balance consists of other hydrocarbons with similar boiling points (primarily C5s). Alternative CAS numbers for the test substance are 68476-43-7, 68527-19-5, 68603-00-9 and 68956-55-8. The three major components analysed were shown to be stable for the duration of the study.
Method	
Method/guideline followed	OECD Method 117 (HPLC Method)
Test type	Partition coefficient
GLP	Yes.
Year	2002
Results	
Partition coefficient	log P = a range between 3.19 and 3.25 at 21°C
Remarks	
Data Quality	Klimish value = 1 (Reliable without restrictions)
Reference:	Huntingdon Life Sciences Ltd. 2002. Physicochemical properties. Project ID CSS035. Huntingdon Life Sciences Ltd., Cambridgeshire, England.
Other	
Last changed	8 June 2004 Robust summary prepared by contractor to Olefins Panel

APPENDIX 3

Robust Summary

Test Substance	
Remarks	Pyrolysis C5s (CAS No. 68476-55-1, hydrocarbons, C5-rich) are produced as a C5 hydrocarbons distillate fraction from pyrolysis gasoline obtained from ethylene production (steam cracking process). The sample tested consisted of isoprene (18%), cis- and trans-pentadiene-1,3 (16%), cyclopentadiene + dicyclopentadiene (14%), n-pentane (10%), cyclopentene (7%), 2-methyl-2-butene (3%) and cyclopentane (1%). The balance consists of other hydrocarbons with similar boiling points (primarily C5s). Alternative CAS numbers for the test substance are 68476-43-7, 68527-19-5, 68603-00-9 and 68956-55-8. The three major components analysed were shown to be stable for the duration of testing in this study.
Method	
Method/guideline followed	Directive 2000/32/EC, B.13/14; OECD Guideline 471; OPPTS 770.5100
GLP	Yes
Year	2002
Type	Bacterial reverse mutation assay
System	Bacterial - *Salmonella typhimurium*, strains TA1535, TA1537, TA98 and TA100 and *E.coli*, strain WP2uvrA/pKM101 (CM891) were used.
Concentrations	0.525, 0.166, 0.0525, 0.0166, 0.00525, 0.00166, 000525 %v/v (5250, 1660, 525, 166, 52.5, 16.6, 5.25 ppm) - test 1 0.525, 0.263, 0.131, 0.066, 0.033%v/v (5250, 2360, 1310, 660, 330 ppm) - test 2
Metabolic activation	With and without S9 mix (10% v/v in test 1, 20% v/v in test 2) prepared from Aroclor-induced rat liver.
Study design	Concentrations of the test substance up to 0.525% v/v (5250 ppm; 50% of the Lower Explosive Limit) were tested in the mutation tests in vapour phase. Agar plates, seeded with the tester strains, were exposed to the test substance for 48 hours at 37°C, then incubated in the absence of the test substance for a further 24 hours. Revertant colony numbers were counted after incubation.

	The following positive control chemicals were used: (a) requiring metabolic activation (response relative to untreated controls): Benzo[a]pyrene, 5 µg/plate: TA98 8-9 x, TA100 3-4 x, TA1537 9-10 x; 2-Aminoanthracene, 2 µg/plate: TA1535 4-13 x; 2-Aminoanthracene, 10 µg/plate: CM891 7-8 x . (b) direct-acting (response relative to untreated controls): 2-Nitrofluorene, 1 µg/plate: TA98 9-23 x; Sodium azide, 0.5 µg/plate: TA100 4-5 x, TA1535 22-27 x; 9-Aminoacridine, 30 µg/plate: TA1537 14-21 x; AF-2, 0.05 µg/plate: CM891 8-9 x; Dichloromethane, 7.5% v/v in vapour phase: TA98 36-51 x; TA100 18 x. All responses were within historical data ranges, except dichloromethane, where responses exceeded the historical data range.
Results	No signs of toxicity observed towards the tester strains in either mutation test. All bacterial lawns were normal. No increases in reversion to prototrophy were obtained in any of the tester strains following exposure to the test substance.
Conclusions	Negative
Data Quality	Valid without restriction
Reference	Huntingdon Life Sciences Ltd. 2002. Bacterial Reverse Mutation Test. Project ID CSS 009. Huntingdon Life Sciences Ltd., Cambridgeshire, England.
Other Last changed	8 September 2004 Robust summary prepared by contractor to Olefins Panel

APPENDIX 7

ROBUST SUMMARY

Test Substance	
Remarks	Pyrolysis C5s (CAS No. 68476-55-1, hydrocarbons, C5-rich) are produced as a C5 hydrocarbons distillate fraction from pyrolysis gasoline obtained from ethylene production (steam cracking process). The sample tested consisted of isoprene (18%), cis- and trans-pentadiene-1,3 (16%), cyclopentadiene + dicyclopentadiene (14%), n-pentane (10%), cyclopentene (7%), 2-methyl-2-butene (3%) and cyclopentane (1%). The balance consists of other hydrocarbons with similar boiling points (primarily C5s). Alternative CAS numbers for the test substance are 68476-43-7, 68527-19-5, 68603-00-9 and 68956-55-8. The three major components analysed were shown to be stable for the duration of testing in this study.
Method	
Method/guideline followed	OECD Guideline 474 (1997), US EPA OPPTS 870.5395 (1998) and EC Directive 2000/32/EC, L 136/50
GLP	yes
Type	Micronucleus assay
Year	2002
Species	mouse
Sex	male
Strain	CD-1
Route of admin.	inhalation
Exposure period	2 x 6 hours approximately 24 hours apart
Doses	40, 125, and 500 parts per million (ppm)
Study design	Groups of seven male CD-1 mice (approximately 32g bodyweight) were exposed to Pyrolysis C5s for two 6-hour whole body exposure periods, on consecutive days, at target concentrations of 40, 150 and 500 (ppm). Compressed air was used to generate atmospheres for both negative control and test chemical exposed groups. Gas chromatography was used to measure the concentrations of Pyrolysis C5s in the test atmospheres. Negative control animals were exposed using compressed air. A positive control group (5 animals) were dosed once only by oral gavage with mitomycin C at 12 mg/kg. All animals were sacrificed approximately 24 hours after the second exposure period (24 hours after the oral dose for the positive control group) and bone marrow smears prepared. Smears were examined to evaluate the incidence of micronuclei (MN) in 2000 polychromatic erythrocytes (PCE) per animal. The proportion of PCE was assessed by examination of at least 1000 erythrocytes.

APPENDIX 7 - continued

Results	No statistically significant increase in the incidence of micronucleated PCE were observed in the Pyrolysis C5s exposed animals compared with the negative control values (Linear-by-Linear association test and exact one-tailed pairwise Permutation test). The positive control treatment induced a significant increase. Statistical significance was declared at the 1% level for all tests.
Conclusion	Negative
Data quality	(1) valid without restriction
Reference:	Huntingdon Life Sciences Ltd. 2002. Mouse Micronucleus Test. Project ID CSS 010. Huntingdon Life Sciences Ltd., Cambridgeshire, England.
Other Last changed	20 April 2004 Robust summary prepared by contractor to Olefins Panel

ROBUST SUMMARIES

Repeated Dose Toxicity

Test Substance	
Remarks	Pyrolysis C5s (CAS No. 68476-55-1, hydrocarbons, C5-rich) are produced from C5 hydrocarbons distillate fraction from pyrolysis gasoline obtained from ethylene production (steam cracking process). The sample tested consisted of isoprene (18%), cis- and trans-pentadiene-1, 3 (16%), cyclopentadiene + dicyclopentadiene (14%), n-pentane (10%), cyclopentene (7%), 2-methyl-2-butene (3%) and cyclopentane (1%). The balance consists of other hydrocarbons with similar boiling point (primarily C5s). Alternative CAS numbers for the test substance are, 68476-43-7, 68527-19-5, 68603-00-9 and 68956-55-8. The three major components analysed were shown to be stable for the duration of testing in this study.
Method	
Method/guideline followed	OECD 422
Test type	4 week general toxicity and reproduction/development toxicity screening test by inhalation exposure to rats (Toxicity phase)
GLP	Yes.
Year	2002
Species	Rat
Strain	Crl:CD® (Sprague-Dawley) IGS BR
Route of administration	Inhalation (gas).
Duration of test	28 days
Doses/concentration levels	0, 98, 302, or 1012 ppm
Sex	12 males, 12 females per dose group for main study group
Exposure period	6 hours/day.
Frequency of treatment	7 days/week
Control group and treatment	12 males, 12 females, air-only exposed.
Post exposure observation period	Not applicable.
Statistical methods	Appropriate statistical analyses were conducted on all parameters.
Test Conditions	Groups of 12 male and 12 female CD rats were exposed to the test material as a gas daily by inhalation for six hours/day at exposure levels of 0, 98, 302, or 1012 ppm. In this main study (repeated-exposure general toxicity) males and females were exposed for 28 days. During the study, clinical condition, detailed functional observational battery, motor activity, bodyweight, food consumption, haematology, blood chemistry, organ weight and macroscopic and microscopic pathology investigations were undertaken. The study also contained reproductive/developmental toxicity satellite groups (summarized separately).
Results	
NOAEL (NOEL)	302 ppm for females, not established for males.
LOAEL (LOEL)	Not applicable.

Remarks	The test atmospheres were analysed by GC and the analysed concentrations were in agreement with the target concentrations. Toxicity Phase There were no signs considered reflective of a general systemic effect observed during routine clinical examination or during the functional observational battery. Occasional intergroup differences in haematological and biochemical parameters were considered to be unrelated to treatment for the following reasons: no correlation between the sexes; no correlation with other changes; slight magnitude of effects all occurring within expected historical ranges; possible atypical control group results. Histopathological changes were restricted to the liver (minimal centrilobular hepatocyte hypertrophy) of High dose rats, associated with slightly higher liver weights. In addition, in male rats, a higher kidney weight and incidence of cortical tubules with hyaline droplets was apparent in all treated groups. High dose males also showed associated kidney lesions. No kidney pathological changes were apparent in treated females.
Conclusions	Slight effects on general systemic toxicity due to the test substance were apparent amongst male animals receiving 98, 302 or 1012 ppm. The no effect level of the test substance for the general systemic toxicity to female rats for 28 days inhalation administration was 302 ppm. A no-effect level for male toxicity was not established.
Data Quality Reliabilities	Klimisch value = 1 (Reliable without restrictions).
References	Huntingdon Life Sciences Ltd., 2004 4-week general toxicity and reproduction/development toxicity screening test by inhalation exposure to rats Project ID CSS 012. Huntingdon Life Sciences Ltd., Cambridgeshire, England.
Other Last changed	7 June 2004 Robust summary prepared by contractor to Olefins Panel

Toxicity to Reproduction

Test Substance	
Remarks	Pyrolysis C5s (CAS No. 68476-55-1, hydrocarbons, C5-rich) are produced from C5 hydrocarbons distillate fraction from pyrolysis gasoline obtained from ethylene production (steam cracking process). The sample tested consisted of isoprene (18%), cis- and trans-pentadiene-1, 3 (16%), cyclopentadiene + dicyclopentadiene (14%), n-pentane (10%), cyclopentene (7%), 2-methyl-2-butene (3%) and cyclopentane (1%). The balance consists of other hydrocarbons with similar boiling point (primarily C5s). Alternative CAS numbers for the test substance are, 68476-43-7, 68527-19-5, 68603-00-9 and 68956-55-8. The three major components analysed were shown to be stable for the duration of testing in this study.
Method	
Method/guideline followed	OECD 422
Test type	4 week general toxicity and reproduction/development toxicity screening test by inhalation exposure to rats (Reproductive phase)
GLP	Yes.
Year	2002
Species	Rat
Strain	Crl:CD® (Sprague-Dawley) IGS BR
Route of administration	Inhalation (gas).
Duration of test	Two weeks prior to breeding, during breeding, and continuing through day 19 of gestation. The dams were then allowed to deliver their litters, which were retained until lactation day 4.
Doses/concentration levels	0, 98, 302 or 1012 ppm
Sex	12 females per dose group for this satellite study.
Exposure period	6 hours/day.
Frequency of treatment	7 days/week
Control group and treatment	12 females, air-only exposed.
Post exposure observation period	Not applicable.
Statistical methods	Appropriate statistical analyses were conducted on all parameters.

Test Conditions	Satellite groups of 12 female Sprague Dawley rats were exposed to the test material as a gas daily by inhalation for six hours/day at exposure levels of 0, 98, 302, or 1012 ppm. The study design included a main study for repeated dose toxicity end points (summarized separately) and reproductive / developmental toxicity satellite groups of 12 females per exposure level. The reproductive/developmental toxicity satellite groups were exposed for two weeks prior to breeding, during breeding and continuing through day 19 of gestation. Males from the main study were used to breed these females. The dams were allowed to deliver their litters, which were retained until lactation Day 4. During the study clinical condition, bodyweight, food consumption, oestrus cycles, mating performance, litter data, organ weights and macroscopic pathology were undertaken.
Results	
NOAEL (NOEL)	1012 ppm.
LOAEL (LOEL)	Not applicable.
Remarks	The test atmospheres were analysed by GC and the analysed concentrations were in agreement with the target concentrations. Reproductive Phase Exposure to female rats for 2 weeks prior to pairing, and up to Day 19 of gestation, did not produce any evidence of any reproduction or developmental toxicity. The oestrous cycle was unaffected by exposure, and mating performance, fertility indices and gestation length were similar in all groups. There were no adverse effects upon survival or growth of the offspring *in uterus* or up to Day 4 of lactation.
Conclusions	The no effect level for reproduction/developmental toxicity was 1012 ppm
Data Quality	
Reliabilities	Klimisch value = 1 (Reliable without restrictions).
References	Huntingdon Life Sciences Ltd., 2004 4-week general toxicity and reproduction/development toxicity screening test by inhalation exposure to rats Project ID CSS 012. Huntingdon Life Sciences Ltd., Cambridgeshire, England.
Other	
Last changed	7 June 2004 Robust summary prepared by contractor to Olefins Panel

APPENDIX 7

ROBUST SUMMARY

Algal growth inhibition assay

Test Substance	
Remarks	Hydrotreated C5s (CAS No. 68602-79-9, distillates, petroleum, benzene unit hydrotreater depentanizer overheads) are Pyrolysis C5s (obtained by distillation from the ethylene production process) that have been partially hydrogenated to reduce diolefin content. The Paraffin/Olefin/Naphthene/Aromatic (PONA) content of the sample tested was 30/61/8/0.1 and the complex mixture consisted of 1,3-butadiene (7%), isopentane (8%), n-pentane (15%), cis- and trans-pentene-2 (12%), 2-methyl-2-butene (8%), cyclopentene (22%), cyclopentane (8%), balance (20%). Alternative CAS numbers for the test substance are, 68410-97-9 and 68603-00-9. The test substance was considered to be stable for the duration of the study.
Method	
Method/guideline followed	OECD Guide-line 201, EC Directive 92/69 C3, US EPA TSCA 797.1050 & 797.1060
GLP	Yes
Year	2002-2003
Endpoint	Growth rate
Species	*Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) (Algae)
Exposure period	96 hours
Limit test	No
Analytical monitoring	Yes
Study design	The study was conducted in completely filled (no headspace) and sealed vessels because of the volatility of Hydrotreated C5s. The test medium was prepared by stirring the test substance in a sealed mixing vessel for approximately 21 hours in the dark. After being allowed to stand for approximately 60 minutes to obtain an equilibrium concentration of the test substance, a portion of the medium was removed from the middle of the vessel and after the inoculation with algal cells, the medium was used to fill replicate test vessels at each concentration. The cultures were incubated in an orbital incubator under continuous illumination at temperatures ranging from 22.0 to 23.2°C for 96 hours. Replicate algal cultures, with an initial cell density of 1 x 10^4/ml, were exposed to Hydrotreated C5s at nominal concentrations of 4.27, 9.39, 20.7, 45.5 and 100 mg/l. The exposure levels were monitored by measuring the concentrations of Hydrotreated C5s in samples of the test media using a GLC method of analysis. Cell densities were measured daily to monitor growth, and the test results are expressed in terms of the area under the growth curve and growth rate.

Evaluation of data	The area under the growth curve and the average specific growth rate are taken to be an index of growth and are calculated mathematically. The E_bL_{50} /E_bC_{50} ("x" h) is the median effect concentration for inhibition of growth based on a comparison of areas under the growth curves after "x" hours. The E_bL_{50} /E_bC_{50} could not be calculated because growth was inhibited by less than 50%. The E_rL_{50} /E_rC_{50} ("x"- "y" h) is the median effect loading rate / concentration for inhibition of growth based on a comparison of growth rates from "x" to "y" hours. The E_rL_{50} /E_rC_{50} could not be calculated because growth was inhibited by less than 50%. The "no observed effect loading rate/ concentration" (NOELR/NOEC) was determined using Dunnett's multicomparison test to compare the percentage inhibition in the test group with that for the control cultures (Dunnett:1955, 1964).
Results	Results for individual measurements were variable, particularly at the lowest level where the measured concentration ranged between 264 and 642% of nominal. As the nominal concentrations increased, the achieved levels decreased, with measured levels of Hydrotreated C5s ranging between 30 and 147% of their nominal values at the start of the test and between 21 and 113% of nominal after 96 hours. Based on a geometric mean, the overall mean measured levels of Hydrotreated C5s were 16.9, 12.1, 13.1, 24.7 and 25.1 mg/l. Failure to achieve exposure levels and variability in the data were thought to reflect the difficulties associated with testing a volatile substance and the varying aqueous solubilities of the constituents. Area under the growth curve: 72 and 96 h E_bL_{50} : > 100 mg/l 72 and 96 h E_bC_{50} : > 25.1mg/l (46% and 43% inhibition at 72 and 96 hours) No observed effect loading rate (NOELR; 96 h) : 9.39 mg/l No observed effect concentratiopn (NOEC; 96 h) : 12.1 mg/l. Average specific growth rate: 72 and 96 h E_rL_{50} : > 100 mg/l 72 and 96 h E_rC_{50} : > 25.1mg/l (6% and 14% inhibition at 72 and 96 hours) No observed effect loading rate (NOELR; 96 h) : 20.7 mg/l No observed effect concentratiopn (NOEC; 96 h) : 13.1 mg/l.
Observations	After 96 hours of exposure, the cells at 45.5 and 100 mg/l (24.7 and 25.1 mg/l, measured) were swollen and/or mis-shapen.

Conclusions	After 96 hours of exposure to Hydrotreated C5s, the E_bL_{50}/E_bC_{50} and E_rL_{50}/E_rC_{50} values were > 100 and >25.1 mg/l respectively. The "no observed effect loading rate / concentration" (NOELR/NOEC) for area under the growth curve respectively were 9.39 and 12.1 mg/l. For growth rate, the NOELR/NOEC respectively were 20.7 and 13.1 mg/l.
Data Quality	Valid without restrictions
Reference:	Huntingdon Life Sciences Ltd. 2002. Algal growth inhibition assay. Project ID CSS 023 Huntingdon Life Sciences Ltd., Cambridgeshire, England
Other Last changed	24 June 2004 Robust summary prepared by contractor to Olefins Panel

APPENDIX 7

ROBUST SUMMARY

Acute Toxicity to *Daphnia Magna*

Test Substance	
Remarks	Hydrotreated C5s (CAS No. 68602-79-9, distillates, petroleum, benzene unit hydrotreater depentanizer overheads) are Pyrolysis C5s (obtained by distillation from the ethylene production process) that have been partially hydrogenated to reduce diolefin content. The Paraffin/Olefin/Naphthene/Aromatic (PONA) content of the sample tested was 30/61/8/0.1 and the complex mixture consisted of 1,3-butadiene (7%), isopentane (8%), n-pentane (15%), cis- and trans-pentene-2 (12%), 2-methyl-2-butene (8%), cyclopentene (22%), cyclopentane (8%), balance (20%). Alternative CAS numbers for the test substance are, 68410-97-9 and 68603-00-9. The test substance was considered to be stable for the duration of the study.
Method	
Method/guideline followed	OECD Guide-line 202 and EC Directive 92/96 C2
GLP	Yes
Year	2002-2003
Test type	Static
Species	*Daphnia magna* (Crustacea)
Exposure period	48 hours
Limit test	No
Analytical monitoring	Yes
Study Design	The study was conducted in completely filled (no headspace) and sealed vessels because of the volatility of Hydrotreated C5s. At each concentration, the test medium was prepared from an aqueous preparation in which the test substance was stirred in a sealed vessel in the dark for approximately 20.5 hours. After being allowed to stand for approximately 1.5 hours to obtain an equilbrium concentration of Hydrotreated C5s, aliquots of medium were removed from the middle of the vessel and used to fill replicate vessels at each concentration. Groups of twenty *Daphnia*, less than 24 hours old, were exposed for 48 hours to Hydrotreated C5s, prepared in Elendt M4 medium at nominal concentrations of 2.13, 4.70, 10.3, 22.7 and 50.0 mg/l. The exposure levels were monitored by measuring the concentrations of Hydrotreated C5s in samples of the test media using a GLC method of analysis. Observations of the *Daphnia* in each control and test vessel were made after 24 and 48 hours.
Evaluation of data	EL_{50} /EC_{50} values were estimated by non-linear interpolation between the two loading rates / concentrations which bracket the 50% effect level using a computer program (Stephan:1977;1982); the program uses the number of *Daphnia* exposed and the number immobile at each nominal loading rate and measured concentration.

Results	Although lower than intended, the mean measured concentrations of Hydrotreated C5s at the start (between 14 and 31% of their nominal values) were adequately maintained during the test, giving measured levels of between 18 and 40% of nominal after 48 hours. Based on a geometric mean, the overall mean measured levels of Hydrotreated C5s were 0.338, 0.783, 3.60, 6.77 and 15.3 mg/l. After 48 hours, the highest loading rate at which no immobilisation occurred was 4.70 mg/l and the lowest loading rate resulting in 100% immobility was 22.7 mg/l; 0.783 and 6.77 mg/l in terms of mean measured Hydrotreated C5s concentrations. 48-hour EL_{50} value = 9.34 mg/l (95% confidence limits of 4.70 and 22.7 mg/l; nominal loading rate) 48-hour EC_{50} value = 2.98 mg/l (95% confidence limits of 0.783 and 6.77 mg/l; measured concentration)
Conclusions	48-hour EL_{50} value = 9.34 mg/l (nominal loading rate) 48-hour EC_{50} value = 2.98 mg/l (measured concentration)
Data Quality	Valid without restrictions
Reference:	Huntingdon Life Sciences Ltd. 2003 Acute Toxicity to *Daphnia magna*. Project ID CSS 024 Huntingdon Life Sciences Ltd., Cambridgeshire, England
Other	
Last changed	22 June 2004 Robust summary prepared by contractor to Olefins Panel

APPENDIX 7

ROBUST SUMARY

Acute Toxicity to Rainbow Trout (Semistatic conditions)

Test Substance	
Remarks	Hydrotreated C5s (CAS No. 68602-79-9, distillates, petroleum, benzene unit hydrotreater depentanizer overheads) are Pyrolysis C5s (obtained by distillation from the ethylene production process) that have been partially hydrogenated to reduce diolefin content. The Paraffin/Olefin/Naphthene/Aromatic (PONA) content of the sample tested was 30/61/8/0.1 and the complex mixture consisted of 1,3-butadiene (7%), isopentane (8%), n-pentane (15%), cis- and trans-pentene-2 (12%), 2-methyl-2-butene (8%), cyclopentene (22%), cyclopentane (8%), balance (20%). Alternative CAS numbers for the test substance are, 68410-97-9 and 68603-00-9. The test substance was considered to be stable for the duration of the study.
Method	
Method/guideline followed	OECD Guide-line 203 and EC Directive 92/96 C1
GLP	Yes
Year	2002
Test type	Semistatic
Species	*Oncorhynchus mykiss* (Rainbow trout; fresh water fish)
Exposure period	96 hours
Limit test	No
Analytical monitoring	Yes
Study Design	The study was conducted in completely filled (no headspace) and sealed vessels because of the volatility of Hydrotreated C5s. At each concentration, the test medium was prepared by stirring the test substance in a sealed vessel for between approximately 20 and 23 hours. After being allowed to stand for at least 60 minutes to obtain an equilibrium concentration of Hydrotreated C5s, aliquots of medium were removed from the middle of the vessel and used to fill the test vessels at each concentration. Groups of ten juvenile fish were exposed for 96 hours to a control solution (dechlorinated tap water, ca. 150-200 mg/l as $CaCO_3$) or to Hydrotreated C5s at nominal concentrations of 2.13, 4.70, 10.3, 22.7 and 50.0 mg/l. The exposure levels were monitored by measuring the concentrations of Hydrotreated C5s in samples of the test media using a GC method of analysis. Observations of the fish were made after 0.25, 2, 4, 24, 48, 72 and 96 hours of exposure.

Evaluation of data	LL_{50}/LC_{50} values were estimated by non-linear interpolation between the two loading rates / concentrations which bracket the 50% effect level using a computer program (Stephan:1977;1982); the program uses the number of fish exposed and the number dead at each nominal loading rate / measured concentration.
Results	The measured concentrations of Hydrotreated C5s ranged between 36% and 79% of their nominal values in samples of freshly prepared media and between 27 and 67% of their nominal values in samples of expired (24-hour-old) media (between 62 and 92% of their starting values). Based on a geometric mean, the overall mean measured levels of Hydrotreated C5s were 1.12, 2.79, 5.33, 10.3 and 19.6 mg/l. After 96 hours, the highest nominal loading rate at which ≤10% mortality had occurred was 4.70 mg/l and the lowest at which there was 100% mortality was 22.7 mg/l; 2.79 and 10.3 mg/l in terms of mean measured Hydrotreated C5s concentrations. Treatment-related effects were exhibited at nominal loading rates of 4.70 mg/l and higher rates (2.79 mg/l, measured). Based on these findings the following values have been estimated: 96-hour LL_{50} value = 10.3 mg/l (95% confidence limits of 4.7 and 22.7 mg/l). 96-hour LC_{50} value = 5.33 mg/l (95% confidence limits of 2.79 and 10.3 mg/l).
Conclusions	96-hour LL_{50} = 10.3 mg/l (loading rate) 96-hour LC_{50} = 5.33 mg/l (measured concentration)
Data Quality ***Reference:***	Valid without restrictions Huntingdon Life Sciences Ltd. 2002. Acute Toxicity to Rainbow Trout (Semi-static exposure conditions). Project ID CSS 025. Huntingdon Life Sciences Ltd., Cambridgeshire, England
Other Last changed	22 June 2004 Robust summary prepared by contractor to Olefins Panel

APPENDIX 9

ROBUST SUMMARY

Test Substance	
Remarks	Hydrotreated C5s (CAS No. 68602-79-9, distillates, petroleum, benzene unit hydrotreater depentanizer overheads) are Pyrolysis C5s (obtained by distillation from the ethylene production process) that have been partially hydrogenated to reduce diolefin content. The Paraffin/Olefin/Naphthene/Aromatic (PONA) content of the sample tested was 30/61/8/0.1 and the complex mixture consisted of 1,3-butadiene (7%), isopentane (8%), n-pentane (15%), cis- and trans-pentene-2 (12%), 2-methyl-2-butene (8%), cyclopentene (22%), cyclopentane (8%), balance (20%). Alternative CAS numbers for the test substance are, 68410-97-9 and 68603-00-9. The test substance was considered to be stable for the duration of the study. The carbon content of the test substance was determined before the start of the Closed Bottle test using a CEC Model 440 Elemental Analyser. The measured carbon content (86.7%) was equivalent to 103% of the theoretical value (84.1%), which was based on the pre-shipment composition of the test substance.
Method	
Method/guideline followed	OECD Guideline 301D and EC Directive 92/69, C.4-E and EPA OPPTS 835.3110.
GLP	Yes
Year	2002
Type	Aerobic
Inoculum	Domestic sewage effluent
Concentration	2 mg/l related to test substance
Contact time	28 days
Test inoculum preparation	A sample of secondary effluent was collected on the day of the test from, a trickling-filter plant, which treats predominantly domestic waste. It was maintained under aerobic conditions in the laboratory, then, immediately before use, filtered through glass wool and the filtrate used as the inoculum for the test (1 ml filtrate/litre test medium).
Study design	Eighteen bottles were filled with a mineral salts medium, inoculated with unacclimated sewage effluent at a loading of 1 ml/l, and the test substance at a nominal loading of 2 mg/l. The dissolved oxygen (DO) concentration in replicate bottles was measured on Days 0, 5, 7, 11, 14, 18, 21, 25 and 28. Four bottles were established for a concurrent five-day microbial inhibition assay, in which the biodegradation of the readily biodegradable reference substance, sodium benzoate, was examined in the presence of the test substance. DO concentration was measured in replicate bottles on Days 0 and 5.

	A further eighteen bottles were filled with mineral salts medium, inoculated with unacclimatised sewage effluent at a loading of 1 ml/l, and the reference substance, sodium benzoate at a nominal loading of 5 mg/l. DO concentrations in replicate bottles was measured on Days 0, 5, 7, 11, 14, 18, 21, 25 and 28. All test systems were incubated at 22 ± 2°C in darkness. Theoretical oxygen demands for the test and reference substances were based on calculated empirical formulae and molecular weights. The study was initiated on 15 October 2002. A sample of secondary effluent was collected on the day of the test from, a trickling-filter plant, which treats predominantly domestic waste. It was maintained under aerobic conditions in the laboratory, then, immediately before use, filtered through glass wool and the filtrate used as the inoculum for the test (1 ml filtrate/litre test medium).
Results	A maximum of 51% biodegradation was measured (on Day 28) by the end of the Closed Bottle test. The mean Total Viable Count of the sample of final sewage effluent in the main test was 1.6 x 10^5 Colony Forming Units (CFU) per ml and was diluted 1000-fold in the medium. The mean count of viable microorganisms in inoculated MSM on Day 0 was 2.7 x 10^3 CFU/ml. The presence of the test substance was not considered to have caused any inhibitory affect on the normal degradative activity of the microbial inoculum on the reference substance.
Degradation Kinetic of test substance	51 (±) % after 28 day(s) 5 day(s) 0 and 0% 7 day(s) 3 and 1% 11 day(s) 0 and 0% 14 day(s) 15 and 28% 18 day(s) 17 and 17% 21 day(s) 33 and 15% 25 day(s) 8 and 30% 28 day(s) 6 and 51%
Control substance Kinetic	Benzoic acid, sodium salt 5 day(s) 76 and 74% 28 day(s) 89 and 88%
Conclusions	Not readily biodegradable
Data Quality	Valid without restriction
Reference	Huntingdon Life Sciences Ltd. 2003. Assessment of biodegradability using the closed bottle method Project ID CSS 026. Huntingdon Life Sciences Ltd., Cambridgeshire, England.
OtherLast changed	14 September 2004 Robust summary prepared by contractor to Olefins Panel

Robust Summary

Biodegradation

Test Substance:	Pyrolysis C5s CAS Number — CAS Inventory Name 68476-55-1[1] — Hydrocarbons, C5-rich 68476-43-7 — Hydrocarbons, C4-6, C5-rich 68527-19-5 — Hydrocarbons, C1-4, debutanizer fraction 68603-00-9 — Distillates, petroleum, thermal cracked naphtha and gas oil 68956-55-8 — Hydrocarbons, C5-unsatd. Note 1: This CAS number has been designated as the suggested CAS number for use in identifying the industry stream in future reporting. Other CAS numbers shown in the list above have been used to identify the stream, and may also be used in the future.
Method/Guideline:	OECD Guideline 301F
Year (guideline):	1992
Type (test type):	Ready Biodegradability: Manometric Respirometry Test
GLP (Y/N):	Yes
Year (study performed):	2003
Inoculum:	Domestic activated sludge
Exposure Period:	28 Days
Test Conditions: • **Note: Concentration preparation, vessel type, replication, test conditions.**	Triplicate test systems were used to evaluate the biodegradability of the test and positive control substances at mean concentrations of 49/L and 46 mg/L, respectively. Blank test systems, which did not contain the test or positive control substance, were run concurrently in triplicate. The total suspended solids (TSS) of the activated sludge was determined to be 4.28 g/L. The inoculum was added at a 1% loading volume of sludge supernatant to test medium. The microbial count of the inoculum was 10^5 CFU/mL. One liter of test medium, which was aerated for 24 hours with carbon dioxide free air, was added to each one liter respirometer flask. The test substance was weighed in an air tight syringe and injected into the test medium. The test system was sealed immediately after addition of the test substance. An aliquot of the positive control stock solution was added to the appropriate test flasks. An unacclimated activated sludge inoculum was used in this study. The inoculum was obtained from the Clinton Sanitary Wastewater Treatment Plant, Annandale, NJ, USA. The treatment plant receives domestic sewage. All test systems were placed on a Coordinated Environmental Services (CES) automated respirometer which automatically recorded the oxygen uptake in general agreement with the OECD guideline. The 28-day study was conducted at a temperature range of 22 ± 1°C.

Results: **Units/Value:** **Note: Deviations from protocol or guideline analytical method.**	Biodegradation was based on oxygen consumption and the theoretical oxygen demand of the test substance as calculated using results of an elemental analysis of the test substance. By day 14, >60% biodegradation of positive control was observed, which meets the guideline requirement. No deviations from the protocol occurred that affected the integrity of the study data. No measurable biodegradation was observed in triplicate test systems therefore the test substance cannot be considered readily biodegradable. Sample — % Degradation* (day 28) — Mean % Degradation (day 28) Test Substance — 0, 0, 0 — 0 Na Benzoate — 91, 100, 89 — 94 * replicate data
Conclusion:	Not readily biodegradable
Reliability:	(1)-Reliable without restriction.
Reference:	ExxonMobil Biomedical Sciences, Inc. 2004. Ready Biodegradability: Manometric Respirometry test. Study # 183994A
Other (source):	Olefins Panel, American Chemistry Council

APPENDIX 5

Robust Summary

PHYSICOCHEMICAL PROPERTIES

Test Substance	
Remarks	Hydrotreated C5s (CAS number 68602-79-9, distillates, petroleum, benzene unit hydrotreater depentanizer overheads) are Pyrolysis C5s (obtained by distillation from the ethylene production process) that have been partially hydrogenated to reduce diolefin content. The Paraffin/Olefin/Napthalene/Aromatic (PONA) content of the sample tested was 30/61/8/0.1 and the complex mixture consisted of 1,3-butadiene (7%), isopentane (8%), n-pentane (15%), cis-and trans-pentene-2 (12%), 2-methyl-2-butene (8%), cyclopentene (22%), cyclopentane (8%), balance (20%). Alternative CAS numbers for the test substance are 68410-97-9 and 68603-00-9. The test substance was stable for the duration of the study.
Method	
Method/guideline followed	OECD Method 103
Test type	Boiling temperature
GLP	Yes.
Year	2002
Results	23.5°C to 52.0°C
Boiling point	Main fractions at 27.5°C and 40.5°C
Remarks	The test substance boils over a temperature range of 23.5°C to 52°C and exhibits two main boiling temperatures of 27.5°C and 40.5°C.
Data Quality	Klimish value = 1 (Reliable without restrictions)
Reference:	Huntingdon Life Sciences Ltd. 2002. Physicochemical properties. Project ID CSS/034. Huntingdon Life Sciences Ltd., Cambridgeshire, England.
Other	
Last changed	19 August 2004 Robust summary prepared by contractor to Olefins Panel

APPENDIX 5 (continued)

PHYSICOCHEMICAL PROPERTIES

Test Substance	
Remarks	Hydrotreated C5s (CAS number 68602-79-9, distillates, petroleum, benzene unit hydrotreater depentanizer overheads) are Pyrolysis C5s (obtained by distillation from the ethylene production process) that have been partially hydrogenated to reduce diolefin content. The Paraffin/Olefin/Napthalene/Aromatic (PONA) content of the sample tested was 30/61/8/0.1 and the complex mixture consisted of 1,3-butadiene (7%), isopentane (8%), n-pentane (15%), cis-and trans-pentene-2 (12%), 2-methyl-2-butene (8%), cyclopentene (22%), cyclopentane (8%), balance (20%). Alternative CAS numbers for the test substance are 68410-97-9 and 68603-00-9. The test substance was stable for the duration of the study.
Method	
Method/guideline followed	OECD Method 104
Test type	Vapour Pressure
GLP	Yes.
Year	2002
Results	
Vapour pressure	82260 Pa at 25°C (617 mm Hg)
Remarks	
Data Quality	Klimish value = 1 (Reliable without restrictions)
Reference:	Huntingdon Life Sciences Ltd. 2002. Physicochemical properties. Project ID CSS/034. Huntingdon Life Sciences Ltd., Cambridgeshire, England.
Other	
Last changed	19 August 2004 Robust summary prepared by contractor to Olefins Panel

APPENDIX 5 (continued)

PHYSICOCHEMICAL PROPERTIES

Test Substance	
Remarks	Hydrotreated C5s (CAS number 68602-79-9, distillates, petroleum, benzene unit hydrotreater dependanizer overheads) are Pyrolysis C5s (obtained by distillation from the ethylene production process) that have been partially hydrogenated to reduce diolefin content. The Paraffin/Olefin/Napthalene/Aromatic (PONA) content of the sample tested was 30/61/8/0.1 and the complex mixture consisted of 1,3-butadiene (7%), isopentane (8%), n-pentane (15%), cis-and trans-pentene-2 (12%), 2-methyl-2-butene (8%), cyclopentene (22%), cyclopentane (8%), balance (20%). Alternative CAS numbers for the test substance are 68410-97-9 and 68603-00-9. The test substance was stable for the duration of the study.
Method	
Method/guideline followed	OECD Method 105 (Flask shake method)
Test type	Water solubility
GLP	Yes.
Year	2002
Results	
Water solubility	0.91 g/l at 20°C
Remarks	
Data Quality	Klimish value = 1 (Reliable without restrictions)
Reference:	Huntingdon Life Sciences Ltd. 2003. Physicochemical properties. Project ID CSS/034. Huntingdon Life Sciences Ltd., Cambridgeshire, England.
Other	
Last changed	8 June 2002 Robust summary prepared by contractor to Olefins Panel

APPENDIX 5 (continued)

PHYSICOCHEMICAL PROPERTIES

Test Substance	
Remarks	Hydrotreated C5s (CAS number 68602-79-9, distillates, petroleum, benzene unit hydrotreater depentanizer overheads) are Pyrolysis C5s (obtained by distillation from the ethylene production process) that have been partially hydrogenated to reduce diolefin content. The Paraffin/Olefin/Napthalene/Aromatic (PONA) content of the sample tested was 30/61/8/0.1 and the complex mixture consisted of 1,3-butadiene (7%), isopentane (8%), n-pentane (15%), cis-and trans-pentene-2 (12%), 2-methyl-2-butene (8%), cyclopentene (22%), cyclopentane (8%), balance (20%). Alternative CAS numbers for the test substance are 68410-97-9 and 68603-00-9. The test substance was stable for the duration of the study.
Method	
Method/guideline followed	OECD Method 117 (HPLC Method)
Test type	Partition coefficient
GLP	Yes.
Year	2002
Results	
Partition coefficient	log P = a range between 2.64 and 4.21 at 21.5°C
Remarks	
Data Quality	Klimish value = 1 (Reliable without restrictions)
Reference:	Huntingdon Life Sciences Ltd. 2002. Physicochemical properties. Project ID CSS/034. Huntingdon Life Sciences Ltd., Cambridgeshire, England.
Other	
Last changed	8 June 2004 Robust summary prepared by contractor to Olefins Panel

APPENDIX 3

Robust Summary

Test Substance	
Remarks	Hydrotreated C5s (CAS No. 68602-79-9, distillates, petroleum, benzene unit hydrotreater depentanizer overheads) are Pyrolysis C5s (obtained by distillation from the ethylene production process) that have been partially hydrogenated to reduce diolefin content. The Paraffin/Olefin/ Naphthene/Aromatic (PONA) content of the sample tested was 30/61/8/0.1 and the complex mixture consisted of 1,3-butadiene (7%), isopentane (8%), n-pentane (15%), cis- and trans-pentene-2 (12%), 2-methyl-2-butene (8%), cyclopentene (22%), cyclopentane (8%), balance (20%). Alternative CAS numbers for the test substance are 68410-97-9 and 68603-00-9. The test substance was considered to be stable for the duration of the study.
Method	
Method/guideline followed	Directive 2000/32/EC, B.13/14; OECD Guideline 471; OPPTS 770.5100
GLP	Yes
Year	2002
Type	Bacterial reverse mutation assay
System	Bacterial - *Salmonella typhimurium*, strains TA1535, TA1537, TA98 and TA100 and *E.coli*, strain WP2uvrA/pKM101 (CM891) were used.
Concentrations	0.85, 0.27, 0.085, 0.027, 0.0085, 0.0027, 00085 %v/v (8500, 2700, 850, 270, 85, 27, 8.5 ppm) - test 1 0.85, 0.425, 0.213, 0.107, 0.054%v/v (8500, 4250, 2130, 1070, 540 ppm) - test 2
Metabolic activation	With and without S9 mix (10% v/v in test 1, 20% v/v in test 2) prepared from Aroclor-induced rat liver.
Study design	Concentrations of the test substance up to 0.85% v/v (8500 ppm; 50% of the Lower Explosive Limit) were tested in the mutation tests in vapour phase. Agar plates, seeded with the tester strains, were exposed to the test substance for 48 hours at 37°C, then incubated in the absence of the test substance for a further 24 hours. Revertant colony numbers were counted after incubation. The following positive control chemicals were used: (a) requiring metabolic activation (response relative to untreated controls): Benzo[a]pyrene, 5 µg/plate: TA98 6-13 x, TA100 4-6 x, TA1537 7-15 x; 2-Aminoanthracene, 2 µg/plate: TA1535 4-16 x; 2-Aminoanthracene, 10 µg/plate: CM891 8-10 x .

	(b) direct-acting (response relative to untreated controls): 2-Nitrofluorene, 1 µg/plate: TA98 9-13 x; Sodium azide, 0.5 µg/plate: TA100 4-6 x, TA1535 16-32 x; 9-Aminoacridine, 50 µg/plate: TA1537 26-37 x; AF-2, 0.05 µg/plate: CM891 3 x; Dichloromethane, 7.5% v/v in vapour phase: TA98 34 x; TA100 9-14 x. All responses were within historical data ranges.
Results	No signs of toxicity observed towards the tester strains in either mutation test. All bacterial lawns were normal. No increases in reversion to prototrophy were obtained in any of the tester strains following exposure to the test substance.
Conclusions	Negative
Data Quality	Valid without restriction
Reference	Huntingdon Life Sciences Ltd. 2002. Bacterial Reverse Mutation Test. Project ID CSS 019. Huntingdon Life Sciences Ltd., Cambridgeshire, England.
Other Last changed	20 April 2004 Robust summary prepared by contractor to Olefins Panel

APPENDIX 7

ROBUST SUMMARY

Test substance	
Remarks	Hydrotreated C5s (CAS No. 68602-79-9, distillates, petroleum, benzene unit hydrotreater depentanizer overheads) are Pyrolysis C5s (obtained by distillation from the ethylene production process) that have been partially hydrogenated to reduce diolefin content. The Paraffin/Olefin/ Naphthene/Aromatic (PONA) content of the sample tested was 30/61/8/0.1 and the complex mixture consisted of 1,3-butadiene (7%), isopentane (8%), n-pentane (15%), cis- and trans-pentene-2 (12%), 2-methyl-2-butene (8%), cyclopentene (22%), cyclopentane (8%), balance (20%). Alternative CAS numbers for the test substance are 68410-97-9 and 68603-00-9. The test substance was considered to be stable for the duration of the study.
Method	
Method/guideline followed	OECD Guideline 474 (1997), US EPA OPPTS 870.5395 (1998) and EC Directive 2000/32/EC, L 136/50
GLP	yes
Year	2002
Type	Micronucleus assay
Species	Mouse
Sex	Male
Strain	CD-1
Route of admin.	Inhalation
Exposure period	2 x 6 hours approximately 24 hours apart
Doses	2000, 4000, and 8000 parts per million (ppm)
Study design	Groups of seven male CD-1 mice (approximately 32g bodyweight) were exposed to Hydrotreated C5s for two 6-hour whole body exposure periods, on consecutive days, at target concentrations of 2000, 4000 and 8000 (ppm). Compressed air was used to generate atmospheres for both negative control and test chemical exposed groups. Gas chromatography was used to measure the concentrations of Hydrotreated C5s in the test atmospheres. Negative control animals were exposed using compressed air. A positive control group (5 animals) were dosed once only by oral gavage with mitomycin C at 12 mg/kg. All animals were sacrificed approximately 24 hours after the second exposure period (24 hours after the oral dose for the positive control group) and bone marrow smears prepared. Smears were examined to evaluate the incidence of micronuclei (MN) in 2000 immature erythrocytes (MIE) per animal. The proportion of MIE was assessed by examination of at least 1000 erythrocytes.

APPENDIX 7 – continued

Results	No statistically significant increase in the incidence of micronucleated MIE were observed in the Hydrotreated C5s exposed animals compared with the negative control values (Linear-by-Linear association test and exact one-tailed pairwise Permutation test). The positive control treatment induced a significant increase. A small statistically significant decrease in the proportion of immature erythrocytes was observed in animals exposed to Hydrotreated C5s. The Jonckheere's test for trend was significant with Groups 1 to 4 included ($P<0.01$). When data from the high exposure group (8000 ppm) was excluded the trend was not significant ($P>0.01$). There were no statistically significant results of the one-tailed Wilcoxon pairwise test for a decrease in the proportion of MIE, from negative control values. The proportion of immature erythrocytes was within the normal range of variability for this species and the decrease was not considered to be of any biological importance. Statistical significance was declared at the 1% level for all tests.
Conclusion	Negative
Data quality	(1) valid without restriction
Reference	Huntingdon Life Sciences Ltd. 2002. Mouse Micronucleus Test. Project ID CSS 020. Huntingdon Life Sciences Ltd., Cambridgeshire, England.
Other Last changed	10 September 2004 Robust summary prepared by contractor to Olefins Panel

APPENDIX 7

ROBUST SUMMARY

Test substance	
Remarks	Hydrotreated C5s (CAS No. 68602-79-9, distillates, petroleum, benzene unit hydrotreater dependentanizer overheads) are Pyrolysis C5s (obtained by distillation from the ethylene production process) that have been partially hydrogenated to reduce diolefin content. The Paraffin/Olefin/ Naphthene/Aromatic (PONA) content of the sample tested was 30/61/8/0.1 and the complex mixture consisted of 1,3-butadiene (7%), isopentane (8%), n-pentane (15%), cis- and trans-pentene-2 (12%), 2-methyl-2-butene (8%), cyclopentene (22%), cyclopentane (8%), balance (20%). Alternative CAS numbers for the test substance are 68410-97-9 and 68603-00-9. The test substance was considered to be stable for the duration of the study.
Method	
Method/guideline followed	OECD Guideline 474 (1997), US EPA OPPTS 870.5395 (1998) and EC Directive 2000/32/EC, L 136/50
GLP	yes
Year	2002
Type	Micronucleus assay
Species	Mouse
Sex	Male
Strain	CD-1
Route of admin.	Inhalation
Exposure period	2 x 6 hours approximately 24 hours apart
Doses	2000, 4000, and 8000 parts per million (ppm)
Study design	Groups of seven male CD-1 mice (approximately 32g bodyweight) were exposed to Hydrotreated C5s for two 6-hour whole body exposure periods, on consecutive days, at target concentrations of 2000, 4000 and 8000 (ppm). Compressed air was used to generate atmospheres for both negative control and test chemical exposed groups. Gas chromatography was used to measure the concentrations of Hydrotreated C5s in the test atmospheres. Negative control animals were exposed using compressed air. A positive control group (5 animals) were dosed once only by oral gavage with mitomycin C at 12 mg/kg. All animals were sacrificed approximately 24 hours after the second exposure period (24 hours after the oral dose for the positive control group) and bone marrow smears prepared. Smears were examined to evaluate the incidence of micronuclei (MN) in 2000 immature erythrocytes (MIE) per animal. The proportion of MIE was assessed by examination of at least 1000 erythrocytes.

APPENDIX 7 – continued

Results	No statistically significant increase in the incidence of micronucleated MIE were observed in the Hydrotreated C5s exposed animals compared with the negative control values (Linear-by-Linear association test and exact one-tailed pairwise Permutation test). The positive control treatment induced a significant increase. A small statistically significant decrease in the proportion of immature erythrocytes was observed in animals exposed to Hydrotreated C5s. The Jonckheere's test for trend was significant with Groups 1 to 4 included ($P<0.01$). When data from the high exposure group (8000 ppm) was excluded the trend was not significant ($P>0.01$). There were no statistically significant results of the one-tailed Wilcoxon pairwise test for a decrease in the proportion of MIE, from negative control values. The proportion of immature erythrocytes was within the normal range of variability for this species and the decrease was not considered to be of any biological importance. Statistical significance was declared at the 1% level for all tests.
Conclusion	Negative
Data quality	(1) valid without restriction
Reference	Huntingdon Life Sciences Ltd. 2002. Mouse Micronucleus Test. Project ID CSS 020. Huntingdon Life Sciences Ltd., Cambridgeshire, England.
Other Last changed	10 September 2004 Robust summary prepared by contractor to Olefins Panel

ROBUST SUMMARIES

Repeated Dose Toxicity

Test Substance	
Remarks	Hydrotreated C5s (CAS No. 68602-79-9, distillates, petroleum, benzene unit hydrotreater dependanizer overheads) are Pyrolysis C5s (obtained by distillation from the ethylene production process) that have been partially hydrogenated to reduce diolefin content. The Paraffin/Olefin/ Naphthene/Aromatic (PONA) content of the sample tested was 30/61/8/0.1 and the complex mixture consisted of 1,3-butadiene (7%), isopentane (8%), n-pentane (15%), cis- and trans-pentene-2 (12%), 2-methyl-2-butene (8%), cyclopentene (22%), cyclopentane (8%), balance (20%). Alternative CAS numbers for the test substance are 68410-97-9 and 68603-00-9. The test substance was considered to be stable for the duration of the study.
Method	
Method/guideline followed	OECD 422
Test type	4 week general toxicity and reproduction/development toxicity screening test by inhalation exposure to rats (Toxicity phase)
GLP	Yes.
Year	2002
Species	Rat
Strain	Crl:CD® (Sprague-Dawley) IGS BR
Route of administration	Inhalation (gas).
Duration of test	28 days
Doses/concentration levels	0, 992, 3033, or 8502 ppm
Sex	12 males, 12 females per dose group for main study group
Exposure period	6 hours/day.
Frequency of treatment	7 days/week
Control group and treatment	12 males, 12 females, air-only exposed.
Post exposure observation period	Not applicable.
Statistical methods	All statistical analyses were carried out separately for males and females. Data relating to food consumption was analysed on a cage basis. For all other parameters, the analyses were carried out using the individual animal as the basic experimental unit. The following data types were analysed at each timepoint separately:- Rearing and activity counts Bodyweight (FOB) and body temperature Grip strength, landing footsplay and motor activity. Bodyweight, using gains over appropriate study periods. Food consumption, over appropriate study periods, using cumulative cage totals. Biochemistry and haematology

	Organ weights, absolute and/or adjusted for terminal bodyweight Pathological findings, for the number of animals with and without each finding. For categorical data, including rearing and activity counts and pathological findings, the proportion of animals was analysed using Fisher's Exact test (Fisher 1973) for each treated group versus the control. For continuous data, Bartlett's test (Bartlett 1937) was first applied to test the homogeneity of variance between the groups. Using tests dependent on the outcome of Bartlett's test, treated groups were then compared with the Control group, incorporating adjustment for multiple comparisons where necessary. The following sequence of statistical tests was used for bodyweight (FOB), body temperature, grip strength, landing footsplay and motor activity, bodyweight, food consumption, organ weight and clinical pathology data. If 75% of the data (across all groups) were the same value, for example c, then a frequency analysis was applied. Treatment groups were compared using a Mantel test for a trend in proportions (Mantel 1963) and also pairwise Fisher's Exact tests (Fisher 1973) for each dose group against the control both for I) values <c versus values >=c, and for ii) values <=c versus values >c, as applicable. If Bartlett's test for variance homogeneity (Bartlett 1937) was not significant at the 1% level, then parametric analysis was applied. If the F1 test for monotonicity of dose-response (Healey 1999) was not significant at the 1% level, Williams' test for a monotonic trend (Williams 1971, 1972) was applied. If the F1 test was significant, suggesting that the dose-response was not monotone, Dunnett's test (Dunnett 1955, 1964) was performed instead. If Bartlett's test was significant at the 1% level, then logarithmic and square-root transformations were tried. If Bartlett's test was still significant, then non-parametric tests were applied. If the H1 test for monotonicity of dose-response (Healey 1999) was not significant at the 1% level, Shirley's test for a monotonic trend (Shirley 1977) was applied. If the H1 test was significant, suggesting that the dose-response was not monotone, Steel's test (Steel 1959) was performed instead. For functional observation battery data and motor activity, pre-treatment values were analysed as detailed above. Data during the treatment phase was analysed using analysis of variance using pre-treatment values as the covariant. For organ weight data, analysis of variance was initially performed using terminal bodyweight as covariate. If the within group relationship between organ weight and bodyweight was significant at the 10% level (Angervall and Carlstrom, 1963), then the treatment comparisons were made on adjusted group means in order to allow for differences in bodyweight which might influence the organ weights.

	Significant differences between Control and treated groups were expressed at the 5% ($p<0.05$) or 1% ($p<0.01$) level. Williams test is denoted by '*'; t tests are denoted by '+', and Shirley's test by '±'.
Test Conditions	Groups of 12 male and 12 female CD rats were exposed to the test material as a gas daily by inhalation for six hours/day at exposure levels of 0, 992, 3033 or 8502 ppm. In this main study (repeated-exposure general toxicity) males and females were exposed for 28 days. During the study, clinical condition, detailed functional observational battery, motor activity, bodyweight, food consumption, haematology, blood chemistry, organ weight and macroscopic and microscopic pathology investigations were undertaken. The study also contained reproductive/developmental toxicity satellite groups (summarized separately).
Results	
NOAEL (NOEL)	992 ppm for females, not established for males (<992 ppm).
LOAEL (LOEL)	3033 ppm for females, <992 ppm for males.
Remarks	The test atmospheres were analysed by GC and the analysed concentrations were in agreement with the target concentrations. Toxicity Phase Salivation was noted in animals receiving 8502 ppm during the treatment period, with lethargy noted on one occasion. Motor activity of males receiving 8502 ppm was reduced throughout the treatment period. Haematocrit and red cell counts were reduced in females exposed to 8502 ppm. Increased kidney weights were noted at 3033 and 8502 ppm, and increased liver weights were noted at 8502 ppm. Histopathological changes included the kidney of male rats (renal cortical tubules with hyaline droplets) with, in all treated males and in females at 3033 and 8502 ppm, an increased incidence of basophilic cortical tubules. Minimal centrilobular hepatocyte hypertrophy of males at 8502 ppm, associated with slightly higher liver weights were noted. In males and females at 8502 ppm and females at 3033 ppm atrophy/disorganisation of the olfactory epithelium of nasal turbinates was noted.

Conclusions	Slight effects on general systemic toxicity due to the test substance were apparent amongst male animals receiving 992, 3033 or 8502 ppm. The no effect level of the test substance for the general systemic toxicity to female rats for 28 days inhalation administration was 992 ppm. A no-effect level for male toxicity was not established (<992 ppm).
Data Quality	
Reliabilities	Klimisch value = 1 (Reliable without restrictions).
References	Huntingdon Life Sciences Ltd., 2004 4-week general toxicity and reproduction/development toxicity screening test by inhalation exposure to rats Project ID CSS 022. Huntingdon Life Sciences Ltd., Cambridgeshire, England.
Other	
Last changed	30 June 2004 Robust summary prepared by contractor to Olefins Panel

Toxicity to Reproduction

Test Substance	
Remarks	Hydrotreated C5s (CAS No. 68602-79-9, distillates, petroleum, benzene unit hydrotreater depentanizer overheads) are Pyrolysis C5s (obtained by distillation from the ethylene production process) that have been partially hydrogenated to reduce diolefin content. The Paraffin/Olefin/ Naphthene/Aromatic (PONA) content of the sample tested was 30/61/8/0.1 and the complex mixture consisted of 1,3-butadiene (7%), isopentane (8%), n-pentane (15%), cis- and trans-pentene-2 (12%), 2-methyl-2-butene (8%), cyclopentene (22%), cyclopentane (8%), balance (20%). Alternative CAS numbers for the test substance are 68410-97-9 and 68603-00-9. The test substance was considered to be stable for the duration of the study.
Method	
Method/guideline followed	OECD 422
Test type	4 week general toxicity and reproduction/development toxicity screening test by inhalation exposure to rats (Reproductive phase)
GLP	Yes.
Year	2002
Species	Rat
Strain	Crl:CD® (Sprague-Dawley) IGS BR
Route of administration	Inhalation (gas).
Duration of test	Two weeks prior to mating, during mating, and continuing through day 19 of gestation. The dams were then allowed to deliver their litters, which were retained until lactation day 4.
Doses/concentration levels	0, 992, 3033, or 8502 ppm
Sex	12 females per dose group.
Exposure period	6 hours/day.
Frequency of treatment	7 days/week
Control group and treatment	12 females, air-only exposed.
Post exposure observation period	Not applicable.
Statistical methods	All statistical analyses were carried out separately for males and females. Data relating to food consumption was analysed on a cage basis. For all other parameters, the analyses were carried out using the individual animal as the basic experimental unit. The following data types were analysed at each timepoint separately:- Bodyweight, using gains over appropriate study periods. Food consumption, over appropriate study periods, using cumulative cage totals. Organ weights, absolute and/or adjusted for terminal bodyweight

	For continuous data, Bartlett's test (Bartlett 1937) was first applied to test the homogeneity of variance between the groups. Using tests dependent on the outcome of Bartlett's test, treated groups were then compared with the Control group, incorporating adjustment for multiple comparisons where necessary. The following sequence of statistical tests was used for bodyweight, food consumption, organ weight and litter data. If 75% of the data (across all groups) were the same value, for example c, then a frequency analysis was applied. Treatment groups were compared using a Mantel test for a trend in proportions (Mantel 1963) and also pairwise Fisher's Exact tests (Fisher 1973) for each dose group against the control both for I) values <c versus values >=c, and for ii) values <=c versus values >c, as applicable. If Bartlett's test for variance homogeneity (Bartlett 1937) was not significant at the 1% level, then parametric analysis was applied. If the F1 test for monotonicity of dose-response (Healey 1999) was not significant at the 1% level, Williams' test for a monotonic trend (Williams 1971, 1972) was applied. If the F1 test was significant, suggesting that the dose-response was not monotone, Dunnett's test (Dunnett 1955, 1964) was performed instead. If Bartlett's test was significant at the 1% level, then logarithmic and square-root transformations were tried. If Bartlett's test was still significant, then non-parametric tests were applied. If the H1 test for monotonicity of dose-response (Healey 1999) was not significant at the 1% level, Shirley's test for a monotonic trend (Shirley 1977) was applied. If the H1 test was significant, suggesting that the dose-response was not monotone, Steel's test (Steel 1959) was performed instead. For organ weight data, analysis of variance was initially performed using terminal bodyweight as covariate. If the within group relationship between organ weight and bodyweight was significant at the 10% level (Angervall and Carlstrom, 1963), then the treatment comparisons were made on adjusted group means in order to allow for differences in bodyweight which might influence the organ weights. Significant differences between Control and treated groups were expressed at the 5% ($p<0.05$) or 1% ($p<0.01$) level. Williams test is denoted by '*'; t tests are denoted by '+', and Shirley's test by '±'.

Test Conditions	Groups of 12 female Sprague Dawley rats were exposed to the test material as a gas daily by inhalation for six hours/day at exposure levels of 0, 992, 3033 or 8502 ppm. The study design included a main study for repeated dose toxicity end points (summarized separately) and reproductive / developmental toxicity satellite groups of 12 females per exposure level. The reproductive/developmental toxicity satellite groups were exposed for two weeks prior to mating, during mating and continuing through day 19 of gestation. Males from the main study were used to breed these females. The dams were allowed to deliver their litters, which were retained until lactation Day 4. During the study clinical condition, bodyweight, food consumption, oestrous cycles, mating performance, litter data, organ weights and macroscopic pathology were undertaken.
Results	
NOAEL (NOEL)	8502 ppm.
LOAEL (LOEL)	Not applicable.
Remarks	The test atmospheres were analysed by GC and the analysed concentrations were in agreement with the target concentrations. Reproductive Phase Exposure to male and female rats for 2 weeks prior to pairing, and for female rats up to Day 19 of gestation, did not produce any evidence of any reproduction or developmental toxicity. The oestrous cycle was unaffected by exposure, and mating performance, fertility indices and gestation length were similar in all groups. There were no adverse effects upon survival or growth of the offspring *in uterus* or up to Day 4 of lactation.
Conclusions	The no effect level for reproductive/developmental toxicity was 8502 ppm
Data Quality	
Reliabilities	Klimisch value = 1 (Reliable without restrictions).
References	Huntingdon Life Sciences Ltd., 2004 4-week general toxicity and reproductive/developmental toxicity screening test by inhalation exposure to rats Project ID CSS 022. Huntingdon Life Sciences Ltd., Cambridgeshire, England.
Other	
Last changed	30 June 2004 Robust summary prepared by contractor to Olefins Panel